Nitrogen Management of Strobilurin-Treated Wheat Crops

Thesis

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Nitrogen Management of Strobilurin-Treated Wheat Crops

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Dedicated to my mother, Mariko Ishikawa

Abstract

Five field experiments were performed in winter wheat crops over three years to study the effects of two strobilurin fungicides, trifloxystrobin and kresoxim-methyl, and different rates of nitrogen on foliar disease severity and the accumulation and partitioning of dry matter and nitrogen. The strobilurin fungicides were included within triazole, epoxiconazole, based fungicide programmes. A few positive interactions were observed between a mixture of triazole and strobilurin and higher nitrogen rates. It was observed that, the crops treated with a mixture of triazole and strobilurin tended to show, compared to those treated with triazole alone, better control of *Septoria* diseases and prolonged green leaf area duration. There was no evidence that aboveground dry matter accumulation was increased following the application of fungicides irrespective of the programme used. However, it was observed in two of the field experiments conducted in the 3rd year, that yield was greater when the plots were treated with a mixture of triazole and strobilurin than when either no fungicide or triazole alone was applied. There was no difference in aboveground nitrogen accumulation between fungicide programmes, however, greater nitrogen accumulation in grains was observed following the application of a mixture of triazole and strobilurin compared to when the plots were treated with either no fungicide or triazole alone. The greater nitrogen accumulation in grains observed with the crops treated with a mixture of triazole and strobilurin appeared to be attributable, more consistently, to an improved translocation of nitrogen to grains than to an increased nitrogen uptake from the soil. The two strobilurins, i.e. kresoxim-methyl and trifloxystrobin, tested in this study, performed differently, most of the time a better performance was observed with trifloxystrobin compared to kresoxim-methyl. Canopy size and accumulation of dry matter and nitrogen were significantly increased by the application of nitrogen fertilizer.

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Introduction

1.1 Background

1.1.1 General

The ultimate aim of agriculture is to harvest solar energy via plants which otherwise human beings cannot utilize directly. Many different agricultural systems have been developed in different parts of the world mainly determined by prevailing climate and topography. Modern agriculture is one of them and its characteristics lie in its reliance on scientific knowledge and its orientation towards market. Thanks to breeding, mechanization and increased use of chemicals such as fertilizers and pesticides, modern agriculture has made it possible to sustain a larger population with fewer people engaged in agriculture. In Europe, the yield per unit of land has doubled and yield per unit of labour has tripled during the period between 1970 and 1993 (Porceddu and Rabbinge, 1997). On the other hand, negative impacts of modern agriculture on the environment have been pointed out ever since Silent Spring was written by Rachel Carson (1962) and rapid depletion of natural resources has become a major concern for many nations and international communities. Sustainable agriculture is now the key phrase that is mentioned everywhere from FAO documents (FAO, 2002) to scientific papers (Hengsdijk, *et al.*, 1998; Smith, *et al.*, 1998), however, accurate definition of this terminology is not very straightforward especially when it comes to operationalization. Further discussions on the definition of 'sustainability' can be found in many sources (FAO, 1992 cited in De Wit *et al.*, 1995;

Loomis and Connor, 1992; Pretty, 1998). At field and farm levels, concepts such as integrated pest management (IPM) (Röling and Van de Fliert, 1998) and integrated crop management (ICM) have been implemented as means to realize sustainable agriculture at the operational level. Field-based studies on crop production whose aim is the practical implementation of their results need to consider various management factors in a balanced manner so that benefits such as increased yields and/or reduced negative impacts on the environment can be obtained under actual farming situations. Although the above description applies to a wide range of crop production, in this thesis, most of the discussion is mentioned in the context of and in reference to wheat, one of the most important crops both in the UK and in the world.

1.1.2 Wheat yield

In most of the wheat growing areas in the world, wheat yield has experienced a substantial increase during the second half of 20th century (Reynolds *et al.*, 1999). Approximately a half of the increase is associated with genetic gain from breeding, while the other half is attributable to improved management practices such as fertilizer use and disease control (Slafer and Andrade, 1991). Although efforts have been made to increase yield and improve quality at the same time by means of breeding, it has to be noted that genetic potential yield is far from full exploitation under current farm practices in many areas (Boyer, 1982). Slafer and Andrade (1991) suspected that the future improvement in crop yields will have to be more dependent on genetic gain rather than technological innovations, as it is expected that competition between industries for limited resources particularly land and water will become more intensive and thus, increased costs required for further technological innovations and implementation under such limited resource use might not be

justified. Despite this speculation for the future, crop management will remain as important as ever. Different varieties respond differently to management practices and environmental conditions and therefore, appropriate production technologies need to be sought for with the introduction of new varieties. In addition, uncertain factors related to production environment such as global warming and soil deterioration would be another reason why management technology needs to be further elaborated.

1.1.3 Foliar diseases in wheat production

Yields of winter wheat can be adversely affected by various diseases caused by different pathogens such as fungi and viruses. Gooding and Davies (1997) estimated that total losses of world wheat production in the absence of crop protection measures would amount to over 50 %. Among diseases that infect various parts of wheat plants, foliar diseases are those that attack leaves and consequently damage both grain yield and quality. In the UK economically significant foliar diseases include *Septoria* diseases, powdery mildew and rust (Polley and Thomas, 1991) caused by fungi. In order to evaluate the effects of diseases on yield, initially efforts were made to relate a measure of disease severity and yield loss as reviewed by Bastiaans (1993). Later on some workers started relating yield to green leaf area (Lim and Gaunt, 1986) based on their view point that crop loss needs to be understood from physiological response of crops to diseases (Madden, 1983). Although reduction of leaf area that is capable of photosynthesis is the apparent symptom of foliar diseases, their effects are not always limited to that. Rabbinge *et al.* (1985) inoculated winter wheat with powdery mildew in the glasshouse and then measured photosynthetic rates under different irradiance and CO_2 concentration levels. They found that powdery mildew caused a strong reduction of the

assimilation rate at light saturation and a tendency to reduce light use efficiency of wheat leaves. Therefore the implication is that, in studying a pathosystem or a system that contains a certain pathosystem, one should be aware of possible complexity as to the way the pathogen affects plants even when an effective tool to quantify the accurate damage to the plants is not available. In order to protect wheat crops from diseases, a combination of various control measures is usually employed including cultural (e.g. crop rotation), genetic (e.g. resistant variety) and chemical (e.g. fungicides) measures of which chemical measure using fungicides will be discussed in the next section.

1.1.4 Fungicides in wheat production

The history of agrochemical use started with Millardet's finding that a mixture of copper sulfate and hydrated lime called Bordeaux mixture effectively control downy mildew (*Plasmopara viticola*) in grape in 1885 (Agrios, 1997). One has to wait until after the World War II to witness the real development of fungicide technology (Neumann, 1997; Hewitt, 1998). Early products were non-systemic protectants, which work on the surface of the plants but do not move inside the plants. Introduction of systemic fungicides revolutionized fungicide discovery and development exploring further yield benefits (Hewitt, 1998). Agrochemical companies established themselves as an indispensable industry for intensive crop production and the competition for the market has been strengthened (Hewitt, 1998). Neumann (1997) compared the number of crop protection companies that hold 75 % of the market share and argued that the share is becoming concentrated into a fewer companies, for example 75 % market shared by 18 companies in 1980 in comparison with 11 companies in 1994 and with recent mergence and acquisition it is even fewer. New

agrochemicals have to meet ever more stringent standard not only for their performance but also for their impact on the environment. Additional positive effects on yield and grain quality apart from protective and curative effects against a wide range of foliar diseases could add extra value to new products. It was in this competitive environment of crop protection industry that a new class of fungicides, the 'strobilurins' was derived from the analogues of a natural product and subsequently developed into products. 'Strobilurins' was derived from Strobilurin A that was found in the Basidiomycete fungi *Strobilurus tenacellus* (Anke *et al.*, 1977). Although Strobilurin A is active *in vitro* against a range of plant pathogenic fungi, its photochemical instability and relatively high volatility result in poor activity *in vivo* (Clough and Godfrey, 1998). Therefore, synthetic programmes were aimed at analogues with improved light stability, low acute mammalian toxicity, appropriate movement properties within the plant and crop safety. The first synthetic strobilurins, azoxystrobin and kresoxim-methyl, were launched in the market in 1996 (Knight *et al.*, 1997). The wheat crops treated with strobilurin fungicides have been reported to show longer green leaf area duration (GLAD) than those treated with conventional fungicides such as triazoles (Jones, 1998) leading to an additional increase in grain yield. Theoretically one can easily associate longer GLAD with prolonged period of leaf photosynthesis, in other words, increased assimilate production. As chlorophyll, the molecule that makes the leaf look green contains significant amounts of N in its chemical composition, some physiological change caused by the application of strobilurin fungicides with respect to plant N budget can also be suspected. Apart from greening effects of fungicides that are sometimes difficult to be clearly separated from the effects of fungicides on diseases, considering that some diseases cause premature leaf senescence of host plants, more distinct physiological changes caused by fungicides including strobilurin

fungicides have been reported as well (Grossman and Retzlaff, 1997; Grossman *et al.*, 1999.) The effects of fungicides that are distinguished from mere disease control are termed 'nontarget effects of fungicides' and discussed by Vyas (1988).

1.1.5 Nitrogen in wheat production

As mentioned previously, N is an important element in the apparatus of assimilate production. In C_3 leaves, up to 50 % of the soluble protein is accounted for by a single enzyme, ribulose 1,5 bisphosphate carboxylase alone (Sinclair and Horie, 1989). Two thirds of chlorophyll is composed of protein (Masuda, 1988). Thus, without surprise one can expect to find a positive relationship be it linear or curvelinear between leaf N concentration and leaf photosynthetic rate (Evans, 1983; Sinclair and Horie, 1989; Evans, 1989; Bindraban, 1997; Dreccer, 1999). N acquisition by plants is crucial in determining the amount of assimilate production and consequently the yield. Apart from physiological importance, N concentration in wheat grain determines utilization and marketability of grains. Bread-making requires between 11-13 % of crude protein concentration at 14 % grain moisture content basis (Gooding and Davies, 1997). N can be applied at various timings and with different methods, in other words, through N application one can manipulate growth, yield and even quality of grains. As N could have significant effects not only on wheat itself but also on pests and diseases that attack wheat, one has to be aware of possible interactions between N management and crop protection management let alone those between management and environmental factors especially soil and weather.

1.1.6 Systems approach and crop growth simulation

Crop production can be theoretically postulated as a function of genetic factors (e.g. varieties), environmental factors (e.g. weather, soil) and management factors (e.g. nutrition management, crop protection management). A systems approach regards crop production as a system, a limited part of reality (De Wit, 1999) and makes it possible to deal with a complex system such as wheat production in a simplified manner. The traditional research method, statistics, is a powerful tool in detecting and explaining quantitative relationships that exist between various factors and output (e.g. yield) of a system and has been used for many years to study crops. However, a statistically obtained relationship is difficult to apply beyond the data sets from which the relationship was derived. In addition, statistics has limits when one attempts to consider crop growth as a dynamic process in time rather than as a static phenomenon (Horie, 1981). Together with a systems approach, crop growth simulation has been developed by a number of workers (Van Keulen and Seligman, 1987; Horie and Nakagawa, 1990; Goudriaan and Van Laar, 1994; Hunt and Pararajasingham, 1995). It attempts to clarify the underlying mechanism as to how various factors affect yield in the process of crop growth. Although one of the aims of developing a crop growth simulation is to predict yield under various environmental and managemental conditions, one should not merely pursue a simulation model that fits well to the observed data sets. It is much more important that the developed model represents scientific disciplines that constitute the system being studied in a straightforward manner rather than a mere good fit. Unnecessarily complicated models require too many data sets and are difficult to use. Modeling is a tool to enhance our understanding of the system we intend to study and should not be regarded as the purpose of the study.

1.2 Aim, Objectives and Approach

1.2.1 Aim of the study

The aim of this study was to understand the interaction between nitrogen and strobilurin fungicides in wheat with respect to canopy development, yield, and nitrogen and carbon assimilation to improve the management of strobilurin-treated wheat crops.

1.2.2 Objectives to achieve the aim of the study

In order to achieve the aim of the study, five objectives were set as the following.

(I) To understand the effects of the use of strobilurin fungicides in regard of fertilizer N rates on the severity of *Septoria* diseases in winter wheat.

(II) To understand the effects of the use of strobilurin fungicides in regard of fertilizer N rates on growth, canopy size, dry matter accumulation, partitioning, yield components and yield of winter wheat.

(III) To understand the effects of the use of strobilurin fungicides in regard of fertilizer N rates on N accumulation and its relation to dry matter accumulation of winter wheat.

(IV) To understand the effects of the use of strobilurin fungicides in regard of fertilizer N rates on morphology of strobilurin-treated winter wheat.

(V) To quantitatively understand the relationship between N accumulation and biomass accumulation of strobilurin-treated winter wheat with the aid of a systems approach and modeling.

1.2.3 Brief description of approach to meet the objectives

A number of field experiments under a factorial design of fungicide programmes and N rates as factors were performed. The fungicide programmes included the use of the triazole, epoxiconazole alone, and in mixture with either kresoxim-methyl or trifloxystrobin, which are strobilurins. A range of N rates were used which varied according to the specific requirements for each field experiment. Variety was added as an extra factor in some of the field experiments. Measurements were taken with respect to the severity of *Septoria* diseases to address the objective (I), dry matter accumulation, yield components and yield unit of land basis to address the objective (II), N accumulation unit of land basis to address the objective (III), plant height and SLA to address the objective (IV). Finally attempts were made to establish a relationship between plant N status and CO_2 assimilation rate (CER) and then to explain biomass accumulation from plant N status to address the objective (V).

1.3 General Materials and Methods

1.3.1 Field experiments

Five field experiments were performed at Harper Adams University College between 1999 and 2002. The field experiments carried out in 1999/2000, 2001 and 2001/2002 are called Cycle I (Photograph 1), Cycle II (Photograph 2) and Cycle III (Photograph 3) respectively. The three field experiments conducted in 2001/2002 are named Cycle III-A, Cycle III-B and Cycle III-C. Layout of field experiments is given in Appendix 1. Soil type is given in Table 1.3.1.1. All the field experiments were designed as factorial of two or three factors (Table 1.3.1.2) with either three (Cycle I and Cycle II) or four replicates (Cycle III). Cycle III-A and Cycle III-C experiments were blocked in two orthogonal directions (double blocking) in order to account for soil variability in both directions. Experiments were randomized block designs with plot of 1.75 m by 10 m in size. Experimental plots were maintained according to standard farm husbandry practices (see Appendix 2) except for fungicide and N applications which were experimental treatments. Hereward, a bread-making variety was used in each of the experiments. In addition, Malacca, another bread-making variety and Equinox, a feed variety were also employed in Cycle II (Table 1.3.1.3). Sowing rate was 350 m^{-2} in all the field experiments except in Cycle III-A where sowing rate was one of the factors (Table 1.3.1.3).

Table 1.3.1.1 Soil type for field experiments

Field Experiment	*Name of field*	*Soil Series*	*Topsoil characteristics*
Cycle I	Buttery Hill	Bridgnorth	Stoneless sandy loam or loamy sand
Cycle II	Flat Nook	Bridgnorth	Stoneless sandy loam or loamy sand
		Newport	Very slightly stony sand loam or loamy sand
Cycle III	Bird's Nest	Salwick	Slightly stony sandy clay loam or sandy loam

Table 1.3.1.2 Treatment factors for field experiments

Experiment	*Variety*	*Sowing rate*	*Fungicide*	*N rates*
Cycle I	NA	NA		
Cycle II		NA		
Cycle III-A	NA		NA	
Cycle III-B	NA	NA		
Cycle III-C		NA		

NA: not applicable

Table 1.3.1.3 Varieties and sowing rates for field experiments

Experiment	*Hereward*	*Malacca*	*Equinox*	*Sowing rates (m^{-2})*
Cycle I		NA	NA	350
Cycle II				350
Cycle III-A		NA	NA	100/400
Cycle III-B		NA	NA	350
Cycle III-C		NA		350

NA: not applicable

1.3.2 Fungicide application

The fungicide treatments are presented in Table 1.3.2. The fungicide programmes consisted of: epoxiconazole (Opus, BASF Ltd; 1 litre ha^{-1}, 12.1 % w/w a.i.); epoxiconazole and kresoxim-methyl (Landmark, BASF Ltd; 1 litre ha^{-1}, 11.5 % w/w a.i.); and a mixture of epoxiconazole and trifloxystrobin (Twist, Novartis Crop Protection UK Ltd; 2 litre ha^{-1}, 12.5 % w/v a.i.). The rate of epoxiconazole was the same for all the fungicide treatments. Fungicides were applied at GS 31 and 39 (Zadoks *et al.*, 1974). Fungicides were applied to each plot using a CO_2 powered hand-held knapsack sprayer. Fungicides were

applied at 200 l ha^{-1} as a medium spray quality. Plant Growth Regulators, Chlormequat chloride and trinexapac ethyl were applied to all the plots at GS 30/31 mixed with fungicides where applicable.

Table 1.3.2 Fungicide programmes employed for field experiments applied at full dose (l ha^{-1})

Field Experiment	*untrt*	*epoxi (Opus)*	*epoxi + kreso (Landmark)*	*epoxi + triflo (Opus + Twist)*
Cycle I		1.0	1.0	1.0 + 2.0
Cycle II		1.0	1.0	1.0 + 2.0
Cycle III-A	NA	NA	NA	NA
Cycle III-B		1.0	NA	1.0 + 2.0
Cycle III-C		1.0	1.0	1.0 + 2.0

untrt: untreated; epoxi: epoxiconazole; kreso: kresoxim-methyl; triflo: trifloxystrobin

NA: not applicable

1.3.3 N application

The rates of N fertilizer were determined by reference to the recommendations by MAFF (Anon., 2000) for the individual experiments in order to follow commercial practice. A variation from this was also used to test the effects of N as well as its interaction with fungicide and variety treatments. The rates of N fertilizer are given in Table 1.3.3. 40 kg ha^{-1} of N was applied at GS 30/31 and the rest at GS 32. Ammonium nitrate (34.5 % N) was broadcast by hand evenly to each plot so that a uniform nitrogen cover was achieved.

Table 1.3.3 Nitrogen rates employed for field experiments (kg ha^{-1})

Field Experiment	*Low*				*High*
Cycle I	0	100	140		
Cycle II		90	130		
Cycle III-A	0	75	150		250
Cycle III-B	0	100	140		
Cycle III-C		100		200	

1.3.4 Determination of Growth Stage

1) Double Ridge Stage

Ten tillers for each variety were subjected to the determination of the double ridge stage by dissecting the stem base with the aid of a microscope. The number of tillers that had reached the double ridge stage as described in the 'Cereal development guide' (Kirby, 1981) was counted. The day when more than 50 % of tillers had reached the double ridge stage was determined to be the double ridge stage of the variety.

2) Growth stage after the applications of treatments

For each plot, the day when the number of tillers that had reached a certain growth stage (Zadoks *et al.*, 1974) exceeded 50 % was determined to be the day of the growth stage.

1.3.5 Sampling

1) Cycle I

Three types of sampling were conducted in Cycle I termed as sampling, LAI sampling and pre-harvest sampling. Sampling was conducted 6 times, while LAI sampling and pre-harvest sampling were conducted once each. In any type of sampling, the border area was always excluded (Fig. 1.3.5). At each sampling, 15 plants (main shoot + tillers) were chosen randomly for each plot and were removed from the soil with roots. At pre-harvest sampling, plants were removed from a known area of 0.0875 m^{-2} at two positions. In both cases, roots were removed in the laboratory. At LAI sampling, plants were removed from a known area of 0.465 m^{-2} at three positions (0.155 m^{-2} for each position).

2) Cycle II, Cycle III-B and Cycle III-C

In the experiments conducted in year 2 and year 3, only 1 type of sampling was conducted and the last sampling was termed pre-harvest sampling. At each sampling, plants were removed from a known area of 0.525 m^{-2} for each plot always excluding the border area (Fig. 1.3.5).

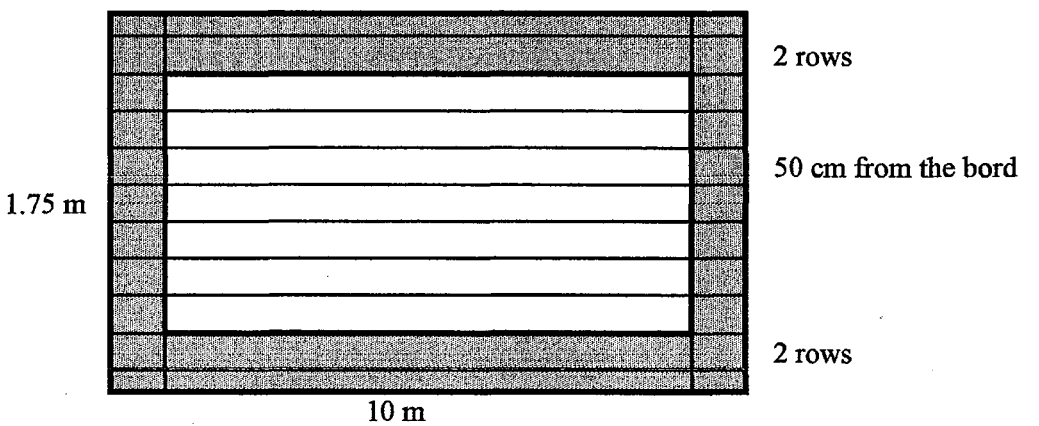


Figure 1.3.5 Discarded area of each plot

1.3.6 Visual Assessment of Foliar Diseases

15 - 20 shoots per plot were subjected to visual foliar disease assessment. For each leaf, the percentage of leaf area covered with the lesions of *Septoria* diseases was visually estimated by interpolating the disease key of Wheat Septoria, Key No. 1.6.1. and 1.6.2 (Anon., 1976). As accurate distinction between *Septoria tritici* and *Septoria nodorum* was not always possible by visual assessment except for the lesions of *Septoria tritici* with the presence of pycnidia, the disease is referred to *Septoria* diseases throughout this study. The same procedure was employed for the assessment of powdery mildew using the disease key of Wheat Mildew, Key No. 1.1.2 (Anon., 1976).

1.3.7 Leaf Area Index (LAI)

Leaf Area Index (LAI) is defined as the sum of the area of all leaves that are green per unit area of ground (Loomis and Connor, 1992). Leaf blade together with leaf collar was removed from the sampled plants (total-sample) from a known area and leaf area was measured for a portion of total-sample (sub-sample) using a delta T Image Analysis System and WinDIAS (Delta-T Devices Ltd). Fresh weight both of total-sample and sub-sample were measured and leaf area of total-sample was calculated from the ratio of fresh weight of total-sample and sub-sample and leaf area of sub-sample. Leaf area of total-sample was divided by the area from which the plants were sampled and leaf area index (LAI) was calculated.

1.3.8 Dry Matter Weight (DMW)

Plant materials were oven-dried at 80 °C for 48 hours. DMW was determined immediately after the removal of the samples from the oven.

1.3.9 Combine Harvest

All the ears of each plot were removed and threshed at the same time by a plot combine either "Haldrup" (2000) or "Wintersteiger" (2001, 2002). Harvested grain weight was measured for each plot and yield was adjusted to 85 % dry matter basis using moisture content explained in section 1.3.10. A sample of 1 kg was retained from the combine-harvested grains of each plot (harvest-sub-sample) for the determination of moisture content and thousand grain weight (TGW).

1.3.10 Moisture Content Determination of Combine-Harvested Grains by Protimeter

Moisture content was estimated using a protimeter, "Grainmaster i" (Protimeter plc) following the manual supplied by the manufacturer.

1.3.11 Moisture Content Determination of Combine-Harvested Grains by Oven Drying

For each plot approximately 100 g of grains from harvest-sub-sample was weighed prior to oven-drying at 80 °C for 48 hours. Dried samples were weighed immediately after they were taken out from the oven. Moisture content was determined using the following equation.

$$MC = \{(GWp - GWo) \times 100\}/(GWp)$$

MC: moisture content (%)

GWp: grain weight prior to oven-drying (g)

GWo: oven-dried grain weight (g)

1.3.12 Thousand Grain Weight (TGW)

Approximately 500 grains from each harvest-sub-sample were counted and weighed. The same procedure was repeated twice. The two values were averaged to obtain TGW of pre-moisture adjustment (TGWp). TGWp was adjusted to 100 % dry matter basis (TGW_{100}) using the following equation.

$TGW_{100} = TGWp \times \{(100 - MC)/100\}$

TGW_{100}: TGW of 100 % dry matter basis

TGWp: TGW of pre-moisture adjustment

MC: moisture content (%)

1.3.13 Mill

Dried plant materials were milled and filtered through 1 mm mesh using a Cyclotec 1093 sample mill (Tecator).

1.3.14 Total N by Kjeldahl

Total N concentration of plant samples was determined in duplicate by the Kjeldahl method (Bremner, 1965a) using a Kjeltec Auto 1030 and 1035 analyzer (Tecator). For each sample, a portion of plant sample was subjected to oven-drying at 100 °C for 24 hours and its moisture content was determined. The results of total N were adjusted to 100 % dry matter basis.

1.3.15 Total N by Dumas

Total nitrogen concentration of plant samples was determined in duplicate using a FP-528 (LECO). The results were adjusted to 100 % dry matter basis by determining moisture content separately in the same procedure described in the previous section 'Total N by Kjeldahl'.

1.3.16 Soil Sampling

Soil samples of 90 cm depth were taken using a set of semi cylindrical gouge augers from each plot. Samples were taken from four positions in each plot of three depths, 0-30 cm, 30-60 cm and 60- 90 cm. The four soil samples from each depth were bulked together and sealed in a plastic bag and kept in a freezer.

1.3.17 Soil Mineral N

The soil samples were passed through a 5.6 mm sieve prior to analysis. Approximately 40g of each soil sample was weighed in a bottle together with 200 ml of the 2M potassium chloride solution (KCl). Each bottle was tightly sealed and shaken on a shaker set at 265 oscillation per minute (O.P.M.) for two hours. Extracted solution was filtered through a Whatman No. 40 filter paper. The filtrate was stored in a fridge until analysis. Total soil mineral N (NH_4^+ + NO_3^-) was determined by steam distillation of the filtered extract with MgO and Devardas alloy (Bremner, 1965b).

1.3.18 CO_2 Exchange Rate (CER) Measurement by IRGA

CO_2 exchange rate (CER) was measured together with other variables such as transpiration rate, photosynthetically active photon flux density (PPFD) and temperature inside the chamber where the measured leaf was enclosed using the Infra-Red Gas Analyzer (IRGA) (PP Systems). Detailed procedures as to measurement are found in Chapter 6 'Photosynthesis and Estimation of Biomass Accumulation'.

1.3.19 Statistical Analysis

Results were analyzed using the Genstat (5th Edition, 6th Edition) statistical programme. ANOVA and regression analysis were performed. All the significant results are referred to at the 5 % probability level. Skeleton analysis of each field experiment is given in Table 1.3.19.

Table 1.3.19 (a) Skeleton analysis of Cycle I

Source of Variation	*Degrees of Freedom*
Block	2
Fungicide	3
N	2
Fungicide* × *N	6
Error	19
Total	32*

*3 missing values

Table 1.3.19 (b) Skeleton analysis of Cycle II

Source of Variation	*Degrees of Freedom*
Block	2
Variety	2
Fungicide	3
N	1
Variety* × *Fungicide	6
Variety* × *N	2
Fungicide* × *N	3
Variety* × *Fungicide* × *N	6
Error	46
Total	71

Table 1.3.19 (c) Skeleton analysis of Cycle III-A

Source of Variation	*Degrees of Freedom*
Block 1	3
Block 2	3
Seed rate	1
N	3
Seed rate × *N*	3
Error	18
Total	31

Table 1.3.19 (d) Skeleton analysis of Cycle III-B

Source of Variation	*Degrees of Freedom*
Block	3
Fungicide	2
N	2
Fungicide × *N*	4
Error	24
Total	35

Table 1.3.19 (e) Skeleton analysis of Cycle III-C

Source of Variation	*Degrees of Freedom*
Block 1	3
Block 2	3
Variety	1
Fungicide	3
N	1
Variety × *Fungicide*	3
Variety × *N*	1
Fungicide × *N*	3
Variety × *Fungicide* × *N*	3
Error	42
Total	63

1.4 Weather Conditions

Daily average temperature and daily precipitation are shown from drilling date to combine-harvest date for each field experimental year (Fig. 1.4 (a), (b), (c)). Daily average temperature was calculated by averaging the maximum temperature and the minimum temperature for each day. The wet conditions in autumn of 2000 delayed the drilling of Cycle II as late as January of 2001. In addition, Cycle II was characterized with a dry spell that prevailed during much of June and prevented the crop from taking up N which led to reduced leaf size (Photograph 4).

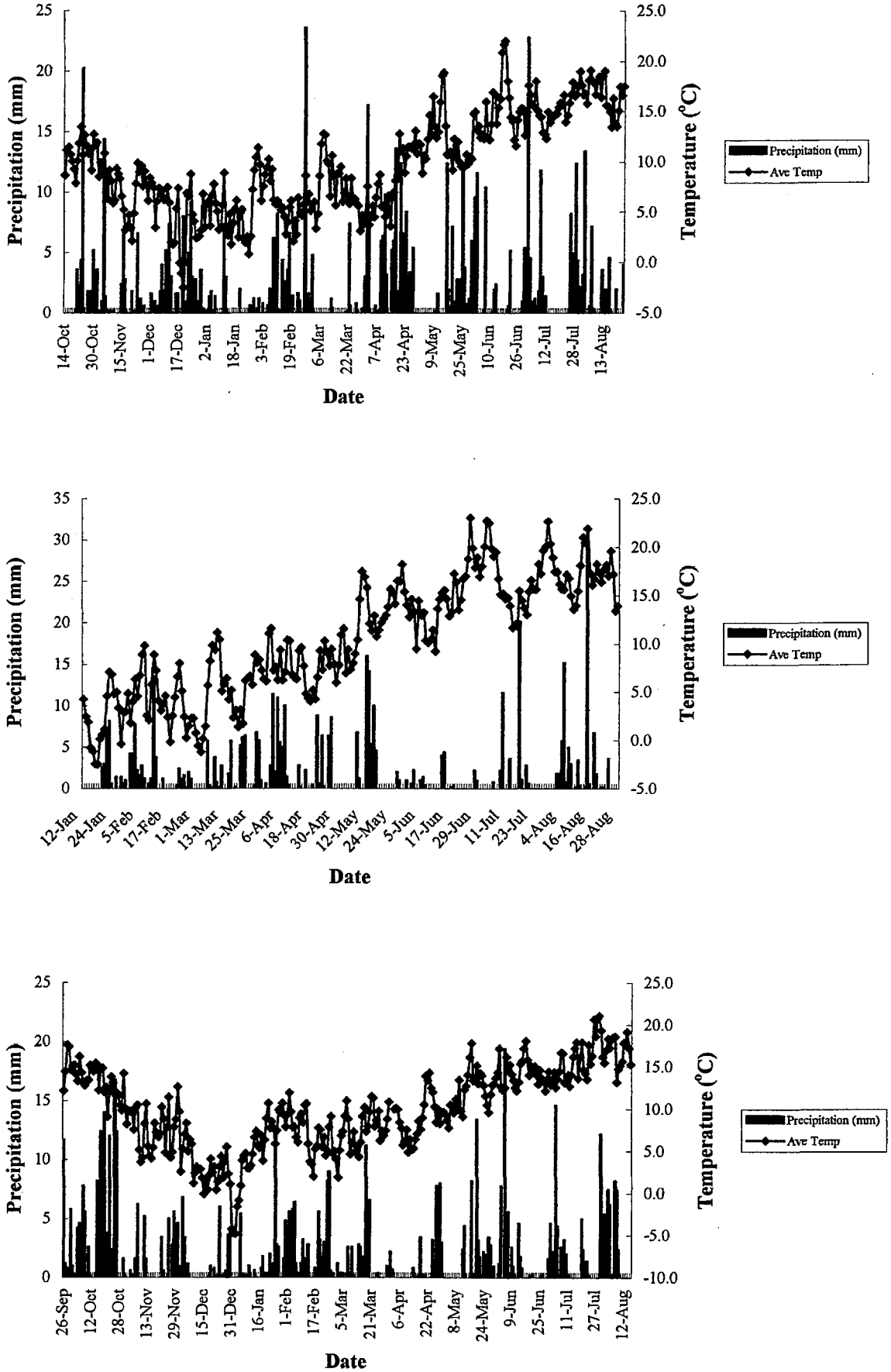


Figure 1.4 Daily average air temperature and daily precipitation in Cycle I (top), Cycle II (middle) and Cycle III (bottom)

Chapter 2

Septoria Diseases

2.1 Introduction

2.1.1 *Septoria* diseases in UK wheat production

Septoria diseases occur throughout the world and affect a number of crops (Agrios, 1997). Leaf blotch (Anamorph: *Septoria tritici*; Telemorph: *Mycosphaerella graminicola*) and glume blotch (Anamorph: *Septoria nodorum*; Telemorph: *Phaeosphaeria nodorum*) of wheat are among them (Agrios, 1997). They are the major foliar diseases that cause serious yield loss in wheat production of England and Wales (Bayles, 1990). The proportions of disease caused by *S. tritici* to those by *S. nodorum* increased greatly during the early 1980s' in the UK (Bayles, 1991). The change of the proportions between the two pathogens is characterized by the development of epidemics of *S. tritici* in summer, which used to happen mostly in winter and early spring followed by a decline in summer (Royle *et al.*, 1986). The widespread occurrence of strains of *S. tritici* resistant to MBC fungicides is suspected to be one of the reasons for later epidemics (Polley and Thomas, 1991), while the prevalent use of susceptible wheat varieties in early 1980s' was identified to be partly responsible (Bayles, 1990).

2.1.2 Symptoms of *Septoria* diseases

Lesions of *S. tritici* are elongate ovals running parallel to leaf veins (Murray *et al.*, 1998). More mature

lesions of *S. tritici* show the presence of black pycnidia (Murray *et al.*, 1998) by which distinction between *S. tritici* and *S. nodorum* is possible. Lesions of *S. nodorum* are ovals coalescing to form large areas of dead brown tissue in which it is difficult to identify brown pycnidia (Murray *et al.*, 1998). A clear distinction between the two *Septoria* diseases in the absence of black pycnidia of *S. tritici* would be difficult with the naked eyes and thus might require a microscopic examination or use of ELISA or PCR.

2.1.3 Mechanism of infection and spread of *Septoria* diseases

Three processes of inoculum transfer of *S. tritici* have been identified by Lovell *et al.* (1997). The first is the wind-mediated-dispersal of ascospores originated from wheat stubble which occurs mainly in autumn and winter (Shaw and Royle, 1989) followed by the primary infections producing pycnidia. The second is the spread of conidial inoculum released from pycnidia mediated by rainsplash in vertical directions (Royle *et al.*, 1986). Sometimes rainsplash is not even needed if an upper leaf layer is in close contact with the lower leaf layer for a period long enough to make inoculum transfer happen between the two layers. In this respect, the factors constituting the crop structure such as rates of leaf emergence and stem elongation influence the efficiency of infection. Lovell *et al.* (1997) investigated the influence of crop structure on the risk of epidemics of *S. tritici* and showed the possibility of new leaves being lower than or at the same height as leaves which are capable of inoculum transfer. This was true particularly with varieties of short statures as previously mentioned by Bahat *et al.* (1980) and by Orth and Grybauskas (1994) with *S. nodorum*. Lovell *et al.* (1997) discussed the effects of crop husbandry on crop canopy and therefore could consider the implications of crop husbandry for disease progress. This shows the importance of considering host,

pathogen and management factors in an integrated manner. The third is the inoculum transfer in horizontal directions. As vertical movement of inoculum by rainsplash by itself is insufficient to cause many lesions in an upper leaf layer, it is likely that the effect of vertical movement is amplified by horizontal movement in the same leaf layer so that even a few primary lesions could cause serious damage (Lovell *et al.*, 1997).

2.1.4 Effects of environmental factors on the disease development

Attempts have been made to identify environmental factors that affect the severity of *Septoria* diseases. Using survey data of wheat crops sampled during 1985-1996 in England, Gladders *et al.* (2001) found that year-to-year variation in severity of *S. tritici* exceeded spatial variability at county levels. This demonstrates the importance of influence of weather and climate factors on performance of this disease. Shaw (1990) reported 40 days at 5 °C as typical latent periods for *S. tritici*, which, with increased temperature, is reduced to 15 days at the optimum of around 15 - 20 °C. A slightly higher range of temperatures (20 - 27 °C) is reported to be optimal for *S. nodorum* (Murray *et al.*, 1998). In summer in the UK, this temperature range could occur commonly and therefore could provide favourable conditions for development of these diseases. In addition to temperature, humidity is another important factor to determine the extent as to the infection of host crops by *Septoria* diseases (Agrios, 1997). Tyldesley and Thompson (1980) found an association between the number of rainy days in the period of mid-May to mid-June and the percentage area of leaf two affected by lesions caused by *S. nodorum* at the milky ripe stage. A wet period at approximately 100 % humidity for 48 hours was considered by several workers to be needed for infection of leaves by *S. tritici* (Shaw, 1991), however, continuous leaf wetness durations of 48 hours or more are not very common

under natural summer conditions in the UK (Shaw, 1990). In a series of experiments held in controlled-environment cabinets, Shaw (1991) observed a substantial infection of wheat leaves by *S.tritici* even under 48 hours of continuous dryness indicating much easier fulfillment for the required conditions to favour the development of the disease.

2.1.5 Effects of N on the expression of *Septoria* diseases

The effect of the state of plant nutrition on the severity of the diseases has been recognized to be important, but few studies have been carried out to investigate the effect of N on the severity of *Septoria* diseases. Contradictory results and statements are found in the literature, although N application is generally considered to increase the severity of *Septoria* diseases (Swain and Melville, 1973; Bayles, 1990; Eyal, 1999). Based on observations made by Thomas (1962) that high N status made wheat seedlings more susceptible to *S. nodorum* infection, Shipton *et al.* (1971) supposed that N applications increase the susceptibility of plants to *S. tritici*.

Prew *et al.* (1983) conducted a field experiment to investigate the relative importance of eight management practices among which N rates and timings were tested on the performance of the wheat crop and applied N of two rates, 160 kg ha^{-1} and 250 kg ha^{-1} respectively, at two application timings, a single dressing in April and a three-way-split in March, April and May. They observed an increased incidence of *Septoria* diseases with greater rates of N. In accordance with their study, Leitch and Jenkins (1995), in two-year field experiments, found increased disease severity by *Septoria* diseases with increased N application rates. They

suggested that the increased incidence of *Septoria* diseases could be induced by the increased nutritional status of the host tissue. On the other hand, Zadoks and Schein (1979) suggested that high N concentrations keep the leaves in a more juvenile state and resistant against *S. nodorum*. Gooding and Davies (1992) mentioned a case where increasing soil application of granular ammonium nitrate led to lower levels of late-season *S. tritici*. Data sets that support such a statement are, however, limited.

Johnston *et al.* (1979) observed an inverse linear relationship between N rates and *Septoria* blotch on spring wheat. Broscious *et al.* (1985) observed such an inverse relationship in one of the 13 field experiments they carried out on farms in Pennsylvania. However, they concluded that the severity of *Septoria* blotch would generally increase as more spring N is applied. In a two-year-field experiment, Arabi *et al.* (2002) observed a decreased severity of infected leaf area by *S. tritici* of up to 38 % following N application. They sought an explanation from Gaunt and Wright (1992) who reasoned that the reduced disease severity relates to the increased carbohydrate storage in leaves and stems following N application. Spink *et al.* (2000) commented that the mechanism as to the way N input affects the severity of *Septoria spp* which are necrotrophic pathogens may differ from that of biotrophs such as powdery mildew and yellow rust. They considered canopy architecture and micro-climate to be more important in determining the severity of *Septoria* diseases.

2.1.6 Effects of *Septoria* diseases on yield, yield components and quality

Most studies have focused on the influence of N on the expansion of the necrotic area or pycnidial coverage on leaf caused by *S. tritici* without paying enough attention to yield and yield component losses (Simon *et al.*, 2002). Forrer and Zadoks (1983) in their inoculation study of *S. tritici* observed a yield reduction that was fully explained by the necrotic leaf area induced by the pathogen, in other words, the reduction of photosynthetically active leaf area. On the other hand, cases have been reported where susceptible varieties of wheat showed insignificant reductions in yield under the severe epidemics of *S. tritici* (Ziv and Eyal, 1978; Zuckerman *et al.*, 1997). It was observed with such a variety that a higher rate of photosynthesis compensated for the reduced photosynthetically active leaf tissues (Zuckerman *et al.*, 1997). Influence of disease on the host is expected to vary depending on the timing and duration of infection. Emphasis is very often placed on infection during grain filling due to its importance in forming yield, however, earlier infection might affect disease development later in the season and consequently yield (Thomson and Gaunt, 1986). Early infection could affect tiller number, spikelet number and floret number as well as grain weight, while effect of infection during grain filling period is limited to reduction in grain weight. Interestingly cases have been observed where poor early growth was compensated by later growth, for example, a decreased grain number was compensated by an increased grain weight (Thomson and Gaunt, 1986), but such compensation could not always be expected (Lim and Gaunt, 1986). As to wheat quality, infection of a susceptible wheat variety by *S. nodorum* increased protein content partly due to yield reduction (Karjalainen and Salovaara, 1988). Similar observations have been reviewed by Dimmock and Gooding (2002). A further investigation as to protein composition might be of interest (Karjalainen and Salovaara,

1988).

2.1.7 Control of *Septoria* diseases

Foliar application of fungicides is the most effective method of disease control for the *Septoria* diseases (Bayles, 1991) as well as selection of resistant varieties (Nelson and Marshall, 1990). Yield-reducing epidemics of *S. tritici* do not usually happen before the emergence of any of the top three leaves (Thomas *et al.*, 1989) and thus fungicide applications should aim to protect these three leaves. Fungicides are not the only chemicals to be applied for controlling *Septoria* diseases. Foliar-applied urea has been observed to reduce *S. nodorum* in wheat when applied at an early stage of infection, although its application after penetration of *Septoria* into plant cells could even enhance disease development (Peltonen *et al.*, 1991). Another form of chemical control, seed treatment of winter wheat with fluquinconazole showed a delay in primary infection by airborne ascospores of *Mycosphaerella graminicola* (Parker and Lovell, 2001). In addition to genetic resistance and chemical control of the diseases, cultural control, for example, disposal of contaminated crop debris and elimination of alternative hosts to harbour pathogens (Murray *et al.*, 1998) may help to reduce incidence of *Septoria* diseases to a lesser extent.

2.1.8 Effects of foliar diseases on crop physiology

The effect of fungal foliar diseases is not always limited to the reduction of photosynthetically active leaf area. For example, Rabbinge *et al.* (1985) inoculated winter wheat with powdery mildew in the glasshouse and then measured photosynthetic rates under different irradiance and CO_2 concentration. They found that

powdery mildew caused a strong reduction of the assimilation rate at light saturation and a tendency to reduce light use efficiency of wheat leaves. Photosynthesis is not the only process affected by foliar diseases. A common observation is that an increase in respiration of the host plant is often induced when infected by pathogenic fungi (Smedegard-Petersen, 1977). In such cases where physiological alterations of host plants are involved, simple measurements of green leaf area would not sufficiently explain yield reduction caused by disease. Studying physiological responses of crops to disease could help us understand the mechanism of yield reduction due to diseases more fully (Madden, 1983). Thomson and Gaunt (1986), for example, reported that infection by *Septoria tritici* affected floret primordium production leading to a reduced grain number per spikelet at all spikelet positions.

2.1.9 Objectives and Hypothesis of Chapter 2

This chapter deals with the Objective (I) in the section of '*1.2 Aim, Objectives and Approach* of Chapter 1' (page 6), i.e., "to understand the effects of the use of strobilurin fungicides in regard of fertilizer N rates on the severity of *Septoria* diseases in winter wheat", two null hypothesis were set.

Firstly this chapter will test the null hypothesis that the use of a strobilurin fungicide in a disease control programme in wheat does not affect disease control. Secondly it will test the null hypothesis that the application of N fertilizer in different rates and consequently the difference in plant N status do not affect the severity of *Septoria* diseases.

Four field experiments under a factorial design of fungicide programmes and N rates as factors were performed. The fungicide programmes included the use of the triazole, epoxiconazole alone, and in mixture with either kresoxim-methyl or trifloxystrobin, which were strobilurins. A range of N rates were used which varied according to the specific requirements for each field experiment. Variety was added as an extra factor in one of the four field experiments.

2.2 Materials and Methods

The data sets from Cycle I, Cycle II, Cycle III-A and Cycle III-B were used in this chapter. Visual assessments of both of the percentage of leaf area covered with lesions of *Septoria* diseases and the percentage of senesced leaf area were conducted using the method described in the section of 1.3.6 in Chapter 1. Assessments were conducted for either 1 or 2 blocks per day, thus it took more than one day to finish taking a set of assessments. Assessment dates are given in Appendix 3. The data sets both of the percentage of leaf area covered with lesions of *Septoria* diseases and that of senesced leaf area were logarithmically transformed using the following equation (Eq. 2.1). The transformed data sets were analyzed with ANOVA and regression analysis using GenStat (5^{th} Edition, 6^{th} Edition). Skeleton analysis of variance for each field experiment is given in the section of 1.3.19 in Chapter 1.

$$Y = \ln (X + 3/8) \quad \text{(Eq. 2.1)}$$

Y: transformed value

X: original value

2.3 Results

Septoria tritici was the dominant disease throughout the four field experiments, although both *S. tritici* and *S. nodorum* were believed to be present. In every experiment, apart from *Septoria* diseases, powdery mildew (*Erysiphe graminis* f. sp. *tritici*) was the only other foliar disease observed at low percentage less than 4 % of leaf area covered with pustules. L.S.D.s are given at 5 % level of *P* value in all figures and tables. In the tables where transformed data are given, the values between brackets are untransformed data.

2.3.1 Relationships between *Septoria* leaf area and senesced leaf area

In Cycle I, only the leaf area covered with lesions of *Septoria* diseases (referred to as *Septoria* leaf area) was recorded. However, later it was considered that, from the view point of crop physiology, what was important to biomass production was senesced leaf area due to its direct relevance to photosynthetically active leaf area and that recording *Septoria* leaf area alone did not seem to provide sufficient information as to the ability of CO_2 assimilation of a crop canopy. In Cycle II and Cycle III, therefore, senesced leaf area as well as *Septoria* leaf area was measured. In this study, senesced leaf area was defined to include *Septoria* leaf area and, therefore, senesced leaf area was always either equal to or greater than *Septoria* leaf area when the former was regressed to the latter (Fig. 2.3.1 (a), (b)). The lines in the graphs (Fig. 2.3.1 (a), (b)) show 1:1 line of *Septoria* leaf area (logarithmically transformed: referred to as LN *Septoria* area) and senesced leaf area (logarithmically transformed: referred to as LN senesced leaf area). Over the three field experiments (Fig. 2.3.1 (a), (b); Table 2.3.1), senesced leaf area showed statistically positive linear relationships to

Septoria leaf area ($P < 0.001$) for the top three leaves within the period, mostly heading and grain-filling period, when visual assessments both of *Septoria* leaf area and senesced leaf area were conducted. In Cycle II, the variability accounted for between the two variables became weaker in the two to three weeks after anthesis except for leaf 1 data that showed the same accountability of 68 %. The degree of reduction in variability accounted for was the greatest for leaf 3 from 76 % down to 26 %. A similar trend was observed in Cycle III-A in which the variability accounted for declined for leaf 3 from 76 % at heading down to 35 % at three weeks after heading. However, in this case the variability accounted for stayed the same for leaf 2 and it increased slightly for leaf 1 during the same period. In Cycle III-B, the variability accounted for between *Septoria* leaf area and senesced leaf area increased by 41 % for leaf 1 and by 20 % for leaf 2 respectively during the period between anthesis and three to four weeks after anthesis. It declined for leaf 3 by 53 % during the same period.

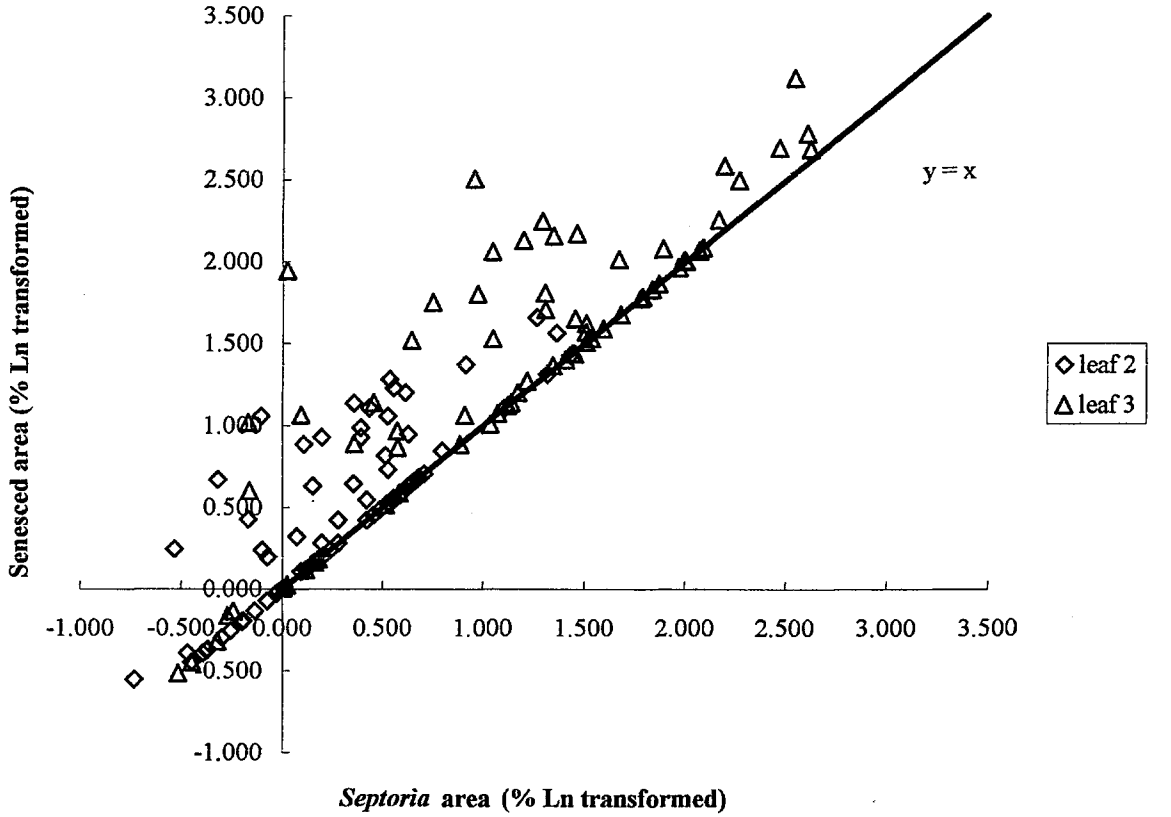


Figure 2.3.1 (a) The relationship between leaf area covered with lesions of *Septoria* diseases and senesced leaf area at anthesis in Cycle II

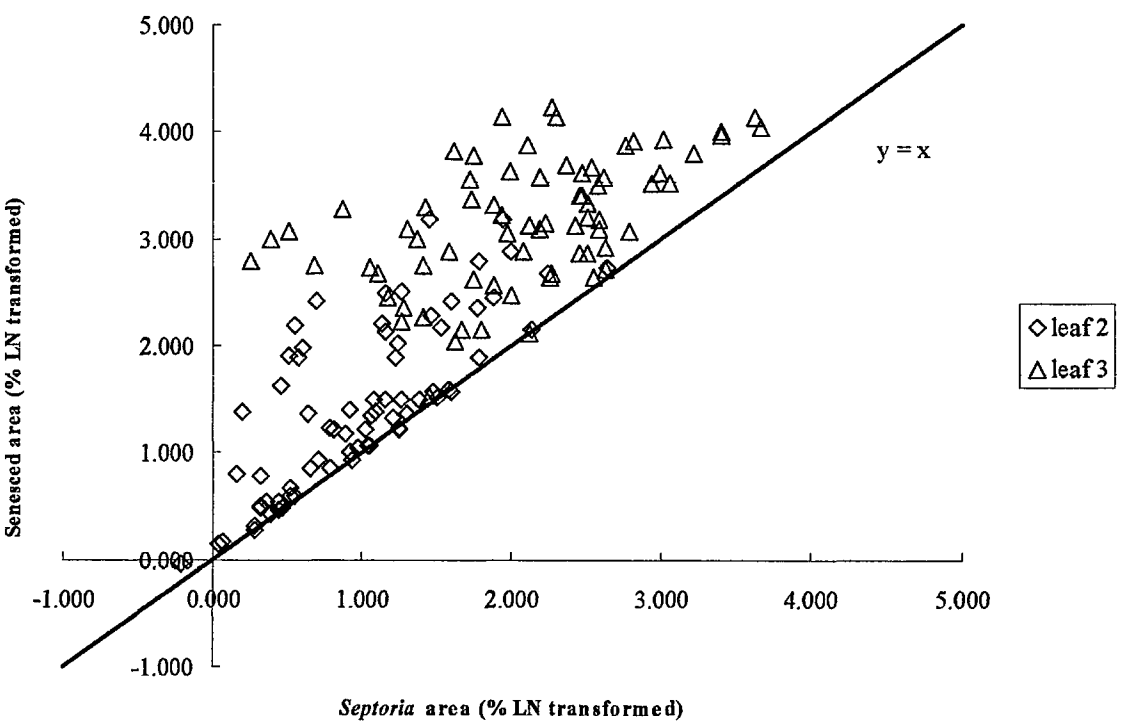


Figure 2.3.1 (b) The relationship between leaf area covered with lesions of *Septoria* diseases and senesced leaf area at approximately three weeks after anthesis in Cycle II

Table 2.3.1 Coefficients of linear regression analysis of the relation between *Septoria* leaf area and senesced leaf area (both LN transformed) for the three field experiments

Cycle	*DAS*	*Leaf*	*Coefficient (t pr.)*	*Constant (t pr.)*	P *value*	R^2
Cycle II	*168-172 (GS 65)*	*Leaf 1*	1.01 (< 0.001)	0.10 (= 0.009)	< 0.001	0.68
		Leaf 2	0.99 (< 0.001)	0.20 (< 0.001)	< 0.001	0.70
		Leaf 3	0.91 (< 0.001)	0.38 (< 0.001)	< 0.001	0.76
	186-193	*Leaf 1*	1.04 (< 0.001)	0.18 (= 0.009)	< 0.001	0.68
		Leaf 2	1.05 (< 0.001)	0.40 (< 0.001)	< 0.001	0.60
		Leaf 3	0.42 (< 0.001)	2.28 (< 0.001)	< 0.001	0.26
Cycle III-A	*251-252 (GS 59)*	*Leaf 1*	1.69 (< 0.001)	0.75 (= 0.005)	< 0.001	0.57
		Leaf 2	0.76 (< 0.001)	0.51 (< 0.001)	< 0.001	0.74
		Leaf 3	0.74 (< 0.001)	0.71 (< 0.001)	< 0.001	0.76
	272-273	*Leaf 1*	0.60 (< 0.001)	1.34 (< 0.001)	< 0.001	0.63
		Leaf 2	1.14 (< 0.001)	0.94 (< 0.001)	< 0.001	0.74
		Leaf 3	1.00 (< 0.001)	1.5 (= 0.022)	< 0.001	0.35
Cycle III-B	*237-244 (GS 49)*	*Leaf 2*	0.90 (< 0.001)	NS (= 0.135)	< 0.001	0.84
		Leaf 3	0.84 (< 0.001)	0.31 (< 0.001)	< 0.001	0.79
	255-264 (GS 65)	*Leaf 1*	0.79 (< 0.001)	0.16 (< 0.001)	< 0.001	0.55
		Leaf 2	0.80 (< 0.001)	0.26 (< 0.001)	< 0.001	0.68
		Leaf 3	0.90 (< 0.001)	0.37 (= 0.001)	< 0.001	0.91
	269-288	*Leaf 1*	1.05 (< 0.001)	0.12 (= 0.039)	< 0.001	0.96
		Leaf 2	0.99 (< 0.001)	0.31 (= 0.037)	< 0.001	0.88
		Leaf 3	0.75 (< 0.001)	1.37 (= 0.012)	< 0.001	0.38

2.3.2 Interactions between treatments in severity of *Septoria* diseases

No interactions between treatments/factors were observed in Cycle I, Cycle III-A and Cycle III-B. In Cycle II, interactions between factors were observed for some of the treatments/factors that had been assessed. An interaction between varieties and fungicide programmes was observed on leaf 1 at 186 – 193 days after sowing (i.e. at approximately three weeks after anthesis) (Fig. 2.3.2 (a)). For Hereward, the plots treated with a mixture of epoxiconazole and trifloxystrobin showed a smaller *Septoria* leaf area than untreated plots by 0.52 % and those treated with a mixture of epoxiconazole and kresoxim-methyl by 0.55 % ($P = 0.043$) (Fig. 2.3.2 (a)). No differences in the severity of *Septoria* diseases between fungicide treatments were observed for Malacca and Equinox. Interactions in severity of *Septoria* diseases between varieties and N rates were observed as well. At 168 – 172 days after sowing (i.e. at anthesis) on leaf 1, Equinox showed a greater *Septoria* leaf area for the plots treated with the N rate of 90 kg ha^{-1} than for those treated with that of 130 kg ha^{-1} by 0.12 %, while no difference between the two N rates was observed for Hereward and Malacca ($P = 0.010$) (Fig. 2.3.2 (b)). The same was observed on leaf 1 at 186 – 193 days after sowing (i.e. at approximately three weeks after anthesis) ($P = 0.032$) (Fig. 2.3.2 (c)).

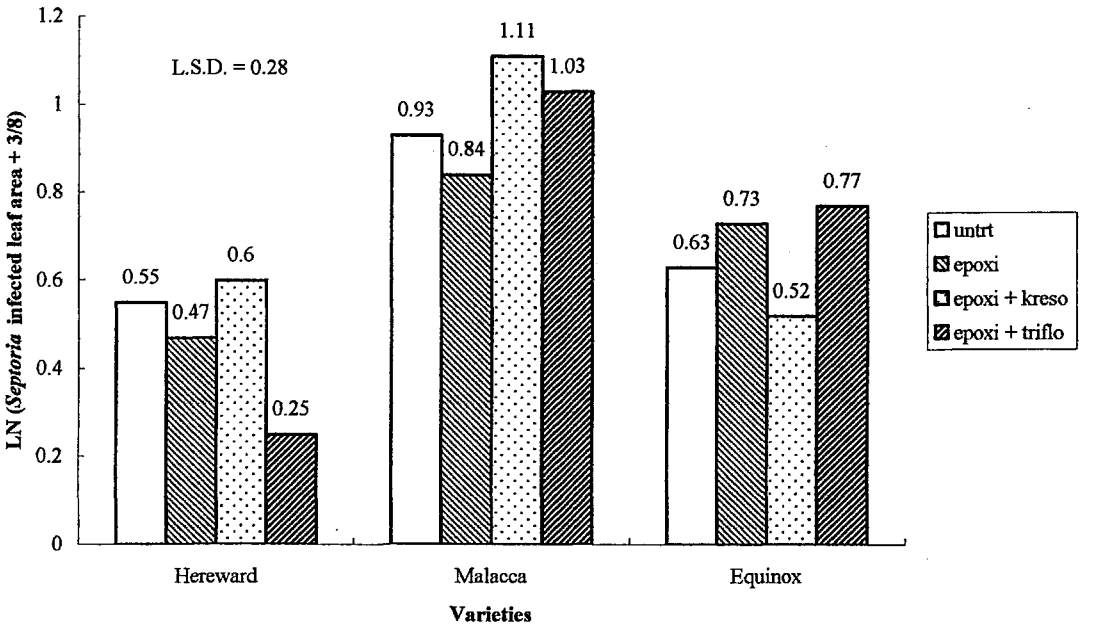


Figure 2.3.2 (a) The interaction between varieties and fungicide programmes in percentage of leaf area (LN transformed) covered with lesions of *Septoria* diseases on leaf 1 at 186 – 193 DAS (at approximately three weeks after anthesis) in Cycle II

untrt: untreated; epoxi: epoxiconazole; kreso: kresoxim-methyl; triflo: trifloxystrobin

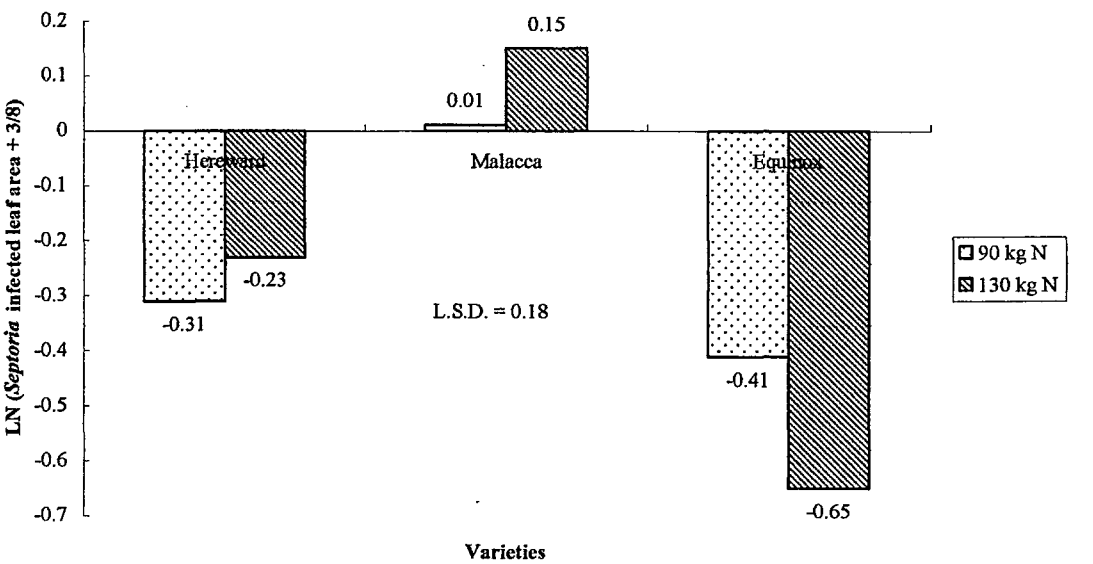


Figure 2.3.2 (b) The interaction between varieties and N rates in percentage of leaf area (LN transformed) covered with lesions of *Septoria* diseases on leaf 1 at 168 – 172 DAS (after anthesis) in Cycle II

kg N: kg N ha^{-1}

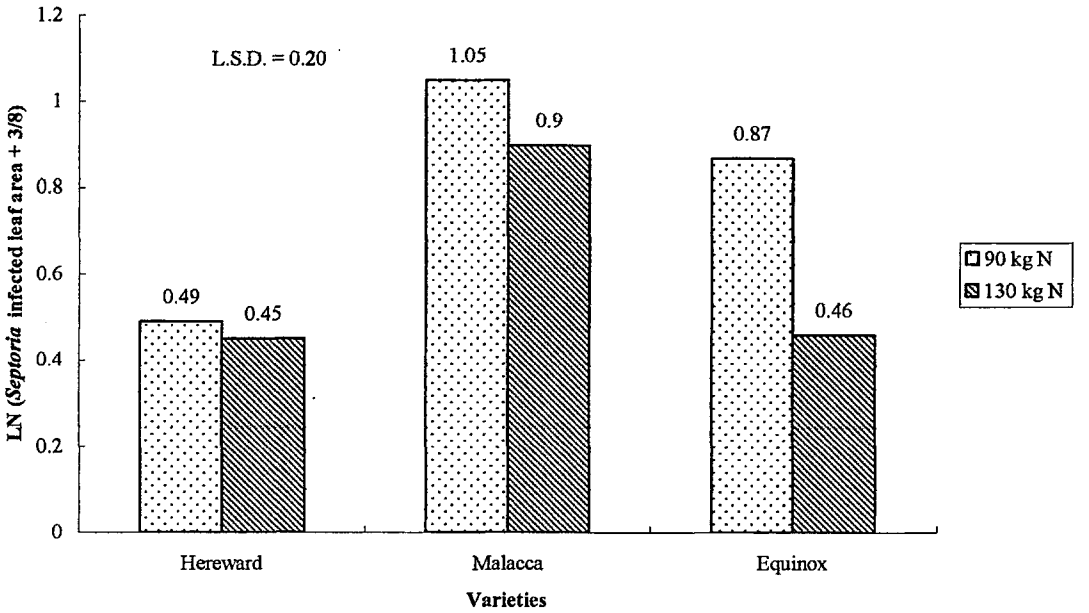


Figure 2.3.2 (c) The interaction between varieties and N rates in percentage of leaf area (LN transformed) covered with lesions of *Septoria* diseases on leaf 1at 186 – 193 DAS (at approximately three weeks after anthesis) in Cycle II

2.3.3 Varieties and severity of *Septoria* diseases

Table 2.3.3 shows the result of disease assessments observed in Cycle II at anthesis and approximately two weeks after anthesis. At 168 - 172 days after sowing (i.e. after anthesis), flag leaf showed only a slight degree of *Septoria* leaf area. The difference between the varieties was significant ($P < 0.001$) with Malacca showing the greatest *Septoria* leaf area and Equinox the smallest. Both on leaf 2 and leaf 3, Hereward showed the greatest *Septoria* leaf area, while Equinox showed the smallest ($P < 0.001$). A similar trend was observed at 186 - 193 days after sowing (i.e. at approximately three weeks after anthesis), although on leaf 2 there was no significant difference in *Septoria* leaf area between varieties.

Table 2.3.3 The percentage of leaf area (LN transformed) covered with lesions of *Septoria* diseases for varieties in Cycle II

DAS	*Leaf*	*Hereward*	*Malacca*	*Equinox*	*L.S.D.*	P *value*	*CV %*
168-172	*Leaf 1*	-0.27	0.08	-0.53	0.13	< 0.001	91.3
(GS 65)		(0.42)	(0.74)	(0.24)			
	Leaf 2	0.64	0.19	-0.07	0.19	< 0.001	127.6
		(1.69)	(0.87)	(0.63)			
	Leaf 3	1.94	1.16	0.23	0.24	< 0.001	36.5
		(7.10)	(3.01)	(1.13)			
186-193	*Leaf 1*	0.47	0.98	0.66	0.14	< 0.001	34.2
		(1.29)	(2.45)	(1.69)			
	Leaf 2	1.1	1.0	0.8	NS	= 0.279	59.1
		(3.0)	(3.0)	(2.5)			
	Leaf 3	2.5	2.1	1.7	0.3	< 0.001	25.7
		(13.8)	(10.0)	(6.3)			

2.3.4 Effects of Seed Rates on Severity of *Septoria* diseases

In Cycle III-A, the only field experiment where seed rate was tested (Photograph 5), *Septoria* leaf area was greater for the seed rate of 400 m^{-2} than that of 100 m^{-2} by 0.23 %, 0.98 % and 3.50 % respectively on leaf 2 ($P = 0.033$), leaf 3 ($P < 0.001$) and leaf 4 ($P < 0.001$) at 251 – 252 days after sowing (i.e. at heading) as well as on leaf 2 ($P = 0.007$) by 1.75 % at 272 – 273 days after sowing (i.e. at approximately two weeks after heading) (Table 2.3.4).

Table 2.3.4 The percentage of leaf area (LN transformed) covered with lesions of *Septoria* diseases for seed rates in Cycle III-A

DAS	*Leaf*	*100 m^{-2}*	*400 m^{-2}*	*L.S.D.*	P *value*	*CV %*
251-252	*Leaf 2*	0.10	0.28	0.16	= 0.033	113.6
(GS 59)		(0.81)	(1.04)			
	Leaf 3	0.97	1.30	0.15	< 0.001	18.1
		(2.62)	(3.60)			
	Leaf 4	1.91	2.34	0.20	< 0.001	12.4
		(7.16)	(10.66)			
272-273	*Leaf 1*	1.44	1.36	NS	= 0.407	20.8
		(4.23)	(3.90)			
	Leaf 2	1.50	1.78	0.20	= 0.007	16.1
		(4.30)	(5.87)			
	Leaf 3	2.48	2.58	NS	= 0.275	10.5
		(12.01)	(13.26)			

2.3.5 Effects of fungicide programmes on severity of *Septoria* diseases

In Cycle I, at 241 – 246 days after sowing (i.e. at anthesis), leaf 2 did not show significant difference in *Septoria* leaf area, while on leaf 3 both untreated plots and those treated with epoxiconazole alone did show a greater *Septoria* leaf area compared to those treated with a mixture of epoxiconazole and kresoxim-methyl by 11.95 % and 4.58 % respectively and a mixture of epoxiconazole and trifloxystrobin by 11.88 % and 4.51 % respectively ($P < 0.001$) (Table 2.3.5 (a)). *Septoria* leaf area was the greatest for untreated plots ($P < 0.001$) (Table 2.3.5 (a)). At 257 – 259 days after sowing (i.e. at approximately two weeks after anthesis), *Septoria* leaf area on leaf 1 did not show any significant difference between fungicide programmes, but on leaf 2 it was greater for untreated plots than those treated with fungicides with the difference ranging from 3.67 % to 5.11 % ($P = 0.010$) (Table 2.3.5 (a)). At 271-273 days after sowing (i.e. at approximately four weeks after

anthesis), *Septoria* leaf area on flag leaf was the greatest for untreated plots and it was lower for the plots treated with a mixture of epoxiconazole and trifloxystrobin than those treated with a mixture of epoxiconazole and kresoxim-methyl by 1.15 % ($P < 0.001$) (Table 2.3.5 (a)).

Table 2.3.5 (a) The percentage of leaf area (LN transformed) covered with lesions of *Septoria* diseases for fungicide programmes in Cycle I

DAS	*Leaf*	*untrt*	*epoxi*	*epoxi + kresoxim*	*epoxi + triflo*	*L.S.D.*	P *value*	*CV %*
241-246 (GS 65)	*Leaf 2*	1.00 (2.51)	0.93 (2.65)	0.92 (2.30)	0.92 (2.27)	NS	= 0.937	31.4
	Leaf 3	2.90 (17.63)	2.32 (10.26)	1.74 (5.68)	1.78 (5.75)	0.28	< 0.001	12.8
257-259	*Leaf 1*	0.52 (1.40)	0.41 (1.16)	0.45 (1.25)	0.38 (1.11)	NS	= 0.460	44.5
	Leaf 2	2.30 (10.74)	1.88 (7.07)	1.81 (6.35)	1.69 (5.63)	0.35	= 0.010	18.5
271-273	*Leaf 1*	2.05 (8.23)	1.57 (4.76)	1.64 (4.88)	1.37 (3.73)	0.26	< 0.001	16.1

untrt: untreated; epoxi: epoxiconazole; kreso: kresoxim-methyl; triflo: trifloxystrobin

In Cycle II, at 168-172 days after sowing (i.e. after anthesis) on flag leaf, both untreated plots and those treated with a mixture of epoxiconazole and kresoxim-methyl showed a greater *Septoria* leaf area compared to those treated with a mixture of epoxiconazole and trifloxystrobin by 0.13 % and 0.20 % respectively ($P = 0.011$) (Table 2.3.5 (b)). The plots treated with a mixture of epoxiconazole and kresoxim-methyl showed a greater *Septoria* leaf area than those treated with epoxiconazole alone by 0.15 % ($P = 0.011$) (Table 2.3.5 (b)). No significant difference was observed on leaf 2 between fungicide programmes (Table 2.3.5 (b)). On leaf

3 *Septoria* leaf area was lower for the plots treated with a mixture of epoxiconazole and trifloxystrobin than those treated with the rest of fungicide programmes with the difference ranging from 0.31 % to 0.48 % (P = 0.008) (Table 2.3.5 (b)). At 186 – 193 days after sowing (i.e. at approximately three weeks after anthesis), no significant difference in *Septoria* leaf area was observed between fungicide programmes (Table 2.3.5 (b)).

Table 2.3.5 (b) The percentage of leaf area (LN transformed) covered with lesions of *Septoria* diseases for fungicide programmes in Cycle II

DAS	*Leaf*	*untrt*	*epoxi*	*epoxi + kresoxim*	*epoxi + triflo*	*L.S.D.*	P *value*	*CV %*
168-172	*Leaf 1*	-0.18	-0.28	-0.13	-0.37	0.15	= 0.011	91.3
(GS 65)		(0.50)	(0.42)	(0.57)	(0.37)			
	Leaf 2	0.34	0.17	0.27	0.24	NS	= 0.495	127.6
		(1.28)	(0.87)	(1.09)	(1.02)			
	Leaf 3	1.31	1.16	1.14	0.83	0.27	= 0.008	36.5
		(4.96)	(3.58)	(3.93)	(2.52)			
186-193	*Leaf 1*	0.70	0.68	0.74	0.68	NS	= 0.849	34.2
		(1.75)	(1.69)	(1.92)	(1.89)			
	Leaf 2	1.1	0.9	1.0	0.9	NS	= 0.642	59.1
		(3.0)	(2.4)	(2.8)	(3.1)			
	Leaf 3	2.4	2.0	2.1	2.0	NS	= 0.099	25.7
		(13.0)	(9.4)	(9.0)	(8.7)			

untrt: untreated; epoxi: epoxiconazole; kreso: kresoxim-methyl; triflo: trifloxystrobin

In Cycle III-B, at 237 – 244 days after sowing (i.e. at approximately two weeks before anthesis), *Septoria* leaf area on leaf 2 was lower for the plots treated with a mixture of epoxiconazole and trifloxystrobin than for untreated plots by 0.21 % and those treated with epoxiconazole alone by 0.11 % (P = 0.001) (Table 2.3.5 (c)). No significant differences were observed both on leaf 3 and leaf 4 between fungicide programmes (Table 2.3.5 (c)). At 255 – 264 days after sowing (i.e. at anthesis), no difference was observed in *Septoria* leaf area

between fungicide programmes both on flag leaf and leaf 4, while untreated plots showed a greater *Septoria* leaf area compared to the plots treated with epoxiconazole alone and those treated with a mixture of epoxiconazole and trifloxystrobin by 0.61 % and 0.48 % respectively on leaf 2 ($P < 0.001$) and by 7.37 % and 5.13 % respectively on leaf 3 ($P = 0.002$) (Table 2.3.5 (c)). At 269 – 288 days after sowing (i.e. at approximately three weeks after anthesis), on flag leaf untreated plots showed a greater *Septoria* leaf area than the plots treated with epoxiconazole alone and those treated with a mixture of epoxiconazole and trifloxystrobin by 6.96 % and 8.52 % respectively ($P < 0.001$) (Table 2.3.5 (c)). On leaf 2 untreated plots showed a greater *Septoria* leaf area compared to the plots treated with epoxiconazole alone and those treated with a mixture of epoxiconazole and trifloxystrobin by 15.87 % and 19.53 % respectively ($P < 0.001$) (Table 2.3.5 (c)). The plots treated with epoxiconazole alone showed a greater *Septoria* leaf area than those treated with a mixture of epoxiconazole and trifloxystrobin by 3.66 % ($P < 0.001$) (Table 2.3.5 (c)). On leaf 3 untreated plots showed a greater *Septoria* leaf area compared to the plots treated with a mixture of epoxiconazole and trifloxystrobin by 15.24 % ($P = 0.030$) (Table 2.3.5 (c)).

Table 2.3.5 (c) The percentage of leaf area (LN transformed) covered with lesions of *Septoria* diseases for fungicide programmes in Cycle III-B

DAS	*Leaf*	*untrt*	*epoxi*	*epoxi + triflo*	*L.S.D.*	P *value*	*CV %*
237-244	*Leaf 2*	-0.38	-0.51	-0.70	0.16	= 0.001	35.0
(GS 49)		(0.35)	(0.25)	(0.14)			
	Leaf 3	0.55	0.49	0.28	NS	= 0.123	73.7
		(1.44)	(1.42)	(1.09)			
	Leaf 4	1.75	1.78	1.58	NS	= 0.340	20.7
		(6.04)	(6.08)	(6.18)			
255-264	*Leaf 1*	0.13	0.04	0.11	NS	= 0.369	168.1
(GS 65)		(0.80)	(0.69)	(0.77)			
	Leaf 2	0.81	0.53	0.60	0.12	< 0.001	22.8
		(1.95)	(1.34)	(1.47)			
	Leaf 3	2.52	1.90	2.05	0.34	= 0.002	18.5
		(14.35)	(6.98)	(9.22)			
	Leaf 4	3.10	2.93	3.23	NS	= 0.068	9.6
		(22.97)	(20.43)	(27.54)			
269-288	*Leaf 1*	1.95	1.35	1.01	0.37	< 0.001	30.2
		(11.03)	(4.07)	(2.51)			
	Leaf 2	2.78	1.87	1.41	0.40	< 0.001	23.3
		(23.88)	(8.01)	(4.35)			
	Leaf 3	3.43	3.19	2.93	0.36	= 0.030	13.6
		(36.66)	(26.55)	(21.42)			

untrt: untreated; epoxi: epoxiconazole; triflo: trifloxystrobin

2.3.6 Effects of N on severity of *Septoria* diseases

In Cycle I, at 241 – 246 days after sowing (i.e. at anthesis), the plots treated with the N rate of 100 kg ha^{-1} and 140 kg ha^{-1} showed a greater *Septoria* leaf area than those that received no N fertilizer by 1.87 % and 1.26 % respectively on leaf 2 ($P < 0.001$) and by 1.96 % and 1.49 % respectively on leaf 3 ($P = 0.039$) (Table 2.3.6 (a)). At 257 – 259 days after sowing (i.e. at approximately two weeks after anthesis), the plots treated

with the N rate of 100 kg ha^{-1} showed a greater *Septoria* leaf area than those treated with the N rate of 0 kg ha^{-1} and 140 kg ha^{-1} by 0.31 % and 0.50 % respectively on flag leaf (P = 0.002) (Table 2.3.6 (a)). On leaf 2, *Septoria* leaf area was the greatest for the plots treated with the N rate of 100 kg ha^{-1} and it was greater for the plots treated with the N rate of 140 kg ha^{-1} than those treated with that of 0 kg ha^{-1} by 2.94 % ($P < 0.001$) (Table 2.3.6 (a)). At 271 – 273 days after sowing (i.e. at approximately four weeks after anthesis), *Septoria* leaf area was smaller for the plots treated with the N rate of 140 kg ha^{-1} than those treated with the other two N rates with the difference ranging from 1.40 % to 1.69 % on flag leaf (P = 0.006) (Table 2.3.6 (a)).

Table 2.3.6 (a) The percentage of leaf area (LN transformed) covered with lesions of *Septoria* diseases for N rates in Cycle I

DAS	*Leaf*	*0 kg N*	*100 kg N*	*140 kg N*	*L.S.D.*	P *value*	*CV %*
241-246	***Leaf 2***	0.53	1.24	1.06	0.25	< 0.001	31.4
(GS 65)		(1.39)	(3.26)	(2.65)			
	Leaf 3	2.00	2.28	2.27	0.24	= 0.039	12.8
		(8.68)	(10.64)	(10.17)			
257-259	***Leaf 1***	0.43	0.62	0.28	0.17	= 0.002	44.5
		(1.19)	(1.50)	(1.00)			
	Leaf 2	1.47	2.31	1.98	0.30	<0.001	18.5
		(4.17)	(10.56)	(7.62)			
271-273	***Leaf 1***	1.83	1.71	1.44	0.23	= 0.006	16.1
		(6.06)	(5.77)	(4.37)			

kg N: kg N ha^{-1}

In Cycle II, the plots treated with the N rate of 90 kg ha^{-1} showed a greater *Septoria* leaf area than those treated with that of 130 kg ha^{-1} by 0.38 % and by 1.05 % respectively on leaf 2 (P = 0.011) and on leaf 3 (P = 0.030) at 168 – 172 days after sowing (i.e. after anthesis) (Table 2.3.6 (b)). Similarly at 186 – 193 days

after sowing (i.e. at approximately three weeks after sowing), *Septoria* leaf area was greater for the plots treated with the N rate of 90 kg ha^{-1} than those treated with that of 130 kg ha^{-1} by 0.46 %, 1.0 % and 3.1 % respectively on flag leaf ($P < 0.001$), leaf 2 ($P = 0.037$) and leaf 3 ($P = 0.008$) (Table 2.3.6 (b)).

Table 2.3.6 (b) The percentage of leaf area (LN transformed) covered with lesions of *Septoria* diseases for N rates in Cycle II

DAS	*Leaf*	*90 kg N*	*130 kg N*	*L.S.D.*	P *value*	*CV %*
168-172	*Leaf 1*	-0.24	-0.24	NS	= 0.964	91.3
(GS 65)		(0.44)	(0.48)			
	Leaf 2	0.36	0.15	0.15	= 0.011	127.6
		(1.26)	(0.88)			
	Leaf 3	1.22	1.00	0.19	= 0.030	36.5
		(4.27)	(3.22)			
186-193	*Leaf 1*	0.80	0.60	0.11	< 0.001	34.2
		(2.04)	(1.58)			
	Leaf 2	1.1	0.8	0.3	= 0.037	59.1
		(3.3)	(2.3)			
	Leaf 3	2.3	1.9	0.3	= 0.008	25.7
		(11.9)	(8.2)			

kg N: kg N ha^{-1}

In Cycle III-A, at 251 – 252 days after sowing (i.e. at heading), *Septoria* leaf area was lower for the plots treated with the N rate of 0 kg ha^{-1} than those treated with the other three N rates on leaf 2 with the difference ranging from 0.50 to 0.78 % ($P < 0.001$) (Table 2.3.6 (c)). Both on leaf 3 ($P < 0.001$) and on leaf 4 ($P < 0.001$), *Septoria* leaf area was the lowest for the plots treated with the N rate of 0 kg ha^{-1} and it was greater for the plots treated with the N rate of 75 kg ha^{-1} than those treated with that of 250 kg ha^{-1} by 0.57 % and 3.16 % respectively (Table 2.3.6 (c)). At 272 – 273 days after sowing (i.e. at approximately two weeks after heading), the plots treated with the N rate of either 150 kg ha^{-1} or 250 kg ha^{-1} showed a greater *Septoria* leaf

area than those treated with that of 0 kg ha^{-1} by 2.19 % and 75 kg ha^{-1} by 2.13 % on flag leaf ($P < 0.001$) (Table 2.3.6 (c)). No difference in *Septoria* leaf area was observed between N rates on leaf 2 and on leaf 3 (Table 2.3.6 (c)).

Table 2.3.6 (c) The percentage of leaf area (LN transformed) covered with lesions of *Septoria* diseases for N rates in Cycle III-A

DAS	*Leaf*	*0 kg N*	*75 kg N*	*150 kg N*	*250 kg N*	*L.S.D.*	P *value*	*CV %*
251-252	*Leaf 2*	-0.26	0.25	0.34	0.43	0.23	< 0.001	113.6
(GS 59)		(0.44)	(0.94)	(1.12)	(1.22)			
	Leaf 3	0.75	1.41	1.22	1.17	0.22	< 0.001	18.1
		(2.04)	(3.79)	(3.39)	(3.22)			
	Leaf 4	1.69	2.41	2.30	2.09	0.28	< 0.001	12.4
		(5.80)	(11.45)	(10.11)	(8.29)			
272-273	*Leaf 1*	1.19	1.10	1.66	1.66	0.31	= 0.001	20.8
		(2.96)	(3.02)	(5.15)	(5.15)			
	Leaf 2	1.51	1.57	1.70	1.77	NS	= 0.211	16.1
		(4.39)	(4.80)	(5.46)	(5.70)			
	Leaf 3	2.54	2.48	2.52	2.58	NS	= 0.275	10.5
		(12.59)	(12.55)	(12.36)	(13.05)			

kg N: kg N ha^{-1}

In Cycle III-B, at 237 – 244 days after sowing (at approximately two weeks before anthesis), the plots treated with the N rate of 0 kg ha^{-1} showed a greater *Septoria* leaf area than those treated with the other two N rates by 0.12 % on leaf 2 ($P = 0.033$), with the difference ranging from 0.64 to 0.87 % on leaf 3 ($P = 0.002$) and from 3.37 to 4.36 % on leaf 4 ($P < 0.001$) (Table 2.3.6 (d)). The same was observed at 255 – 264 days after sowing (i.e. at anthesis) with the difference ranging from 4.35 % to 5.39 % on leaf 3 ($P = 0.005$) and from 10.11 % to 14.26 % on leaf 4 ($P < 0.001$), while on flag leaf *Septoria* leaf area was greater for the plots treated with the N rate of 0 kg ha^{-1} than for those treated with that of 140 kg ha^{-1} by 0.20 % ($P = 0.012$) and

on leaf 2 no difference in *Septoria* leaf area between N rates was observed (Table 2.3.6 (d)). At 269 – 288 days after sowing (i.e. at approximately three weeks after anthesis), *Septoria* leaf area was lower for the plots treated with the N rate of 0 kg ha^{-1} than those treated with the other two N rates with the difference ranging from 0.44 % to 0.56 % on leaf 3 ($P = 0.036$), but no difference was observed between N rates on leaf 1 and on leaf 2 (Table 2.3.6 (d)).

Table 2.3.6 (d) The percentage of leaf area (LN transformed) covered with lesions of *Septoria* diseases for N rates in Cycle III-B

DAS	*Leaf*	*0 kg N*	*100 kg N*	*140 kg N*	*L.S.D.*	P *value*	*CV %*
237-244	*Leaf 2*	-0.65	-0.46	-0.47	0.16	= 0.033	35.0
(GS 49)		(0.16)	(0.28)	(0.28)			
	Leaf 3	0.14	0.61	0.57	0.27	= 0.002	73.7
		(0.83)	(1.65)	(1.47)			
	Leaf 4	1.22	2.00	1.88	0.30	< 0.001	20.7
		(3.43)	(8.06)	(6.80)			
255-264	*Leaf 1*	0.20	0.08	-0.01	0.13	= 0.012	168.1
(GS 65)		(0.87)	(0.73)	(0.67)			
	Leaf 2	0.66	0.60	0.67	NS	= 0.424	22.8
		(1.60)	(1.46)	(1.70)			
	Leaf 3	1.82	2.29	2.37	0.34	= 0.005	18.5
		(6.94)	(11.29)	(12.33)			
	Leaf 4	2.71	3.21	3.34	0.25	< 0.001	9.6
		(15.52)	(25.63)	(29.78)			
269-288	*Leaf 1*	1.33	1.57	1.42	NS	= 0.423	30.2
		(4.28)	(7.26)	(6.07)			
	Leaf 2	1.86	2.18	2.02	NS	= 0.266	23.3
		(10.30)	(15.27)	(10.68)			
	Leaf 3	2.85	3.29	3.41	0.36	= 0.010	13.6
		(21.17)	(30.92)	(32.54)			

kg N: kg N ha^{-1}

2.3.7 Relationships between leaf N concentration and *Septoria* leaf area

From the section 2.3.6, it was learned that rates of N application had significant influence on *Septoria* leaf area throughout the four field experiments suggesting a possibility that looking at N status of plants might provide further information as to the relationship between the rate of N application and severity of *Septoria* diseases. Either a linear or a quadratic equation (Eq. 2.2) was fitted for the relationships between leaf N concentration and *Septoria* leaf area. Mean value of each treatment was employed for these analyses.

$$Y = a X^2 + b X + c \qquad \text{(Eq. 2.2)}$$

Y: *Septoria* leaf area (logarithmically transformed)

X: Leaf N concentration (%)

In Cycle I, a quadratic equation explained well the relationship between leaf N concentration and *Septoria* leaf area on leaf 2 ($P = 0.001$) at 241 – 246 days after sowing (i.e. at anthesis), both on leaf 1 ($P = 0.004$) and on leaf 2 ($P = 0.006$) at 257 – 259 days after sowing (i.e. at approximately two weeks after anthesis) (Fig. 2.3.7 (a), (b), Table 2.3.7 (a)).

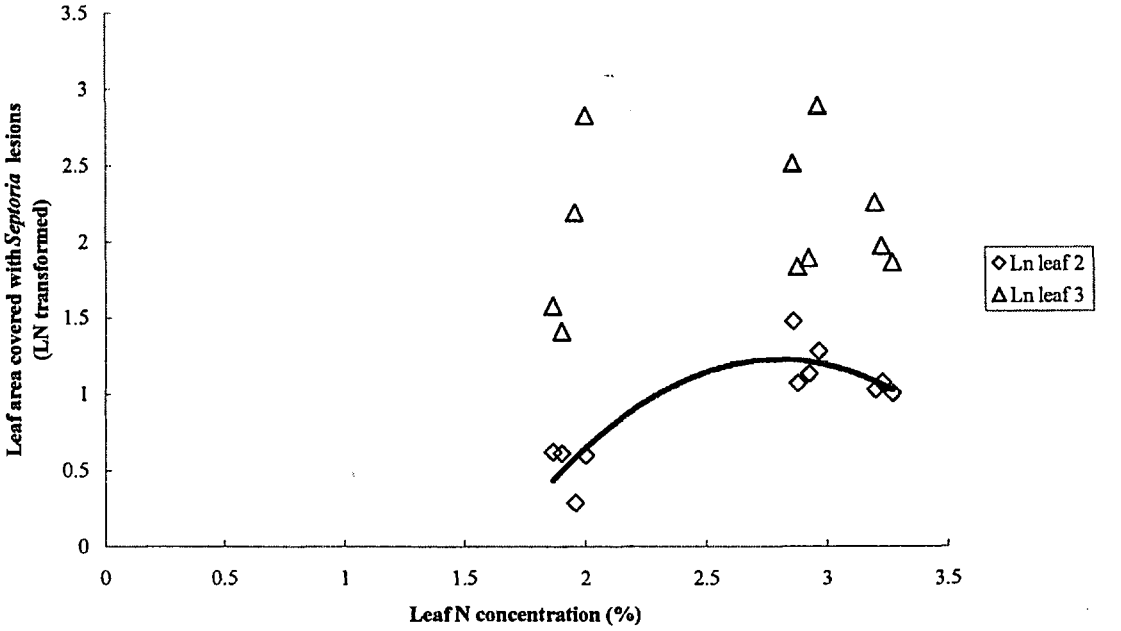


Figure 2.3.7 (a) The relationship between leaf N concentration and *Septoria* leaf area at 241 – 246 DAS (at anthesis) in Cycle I

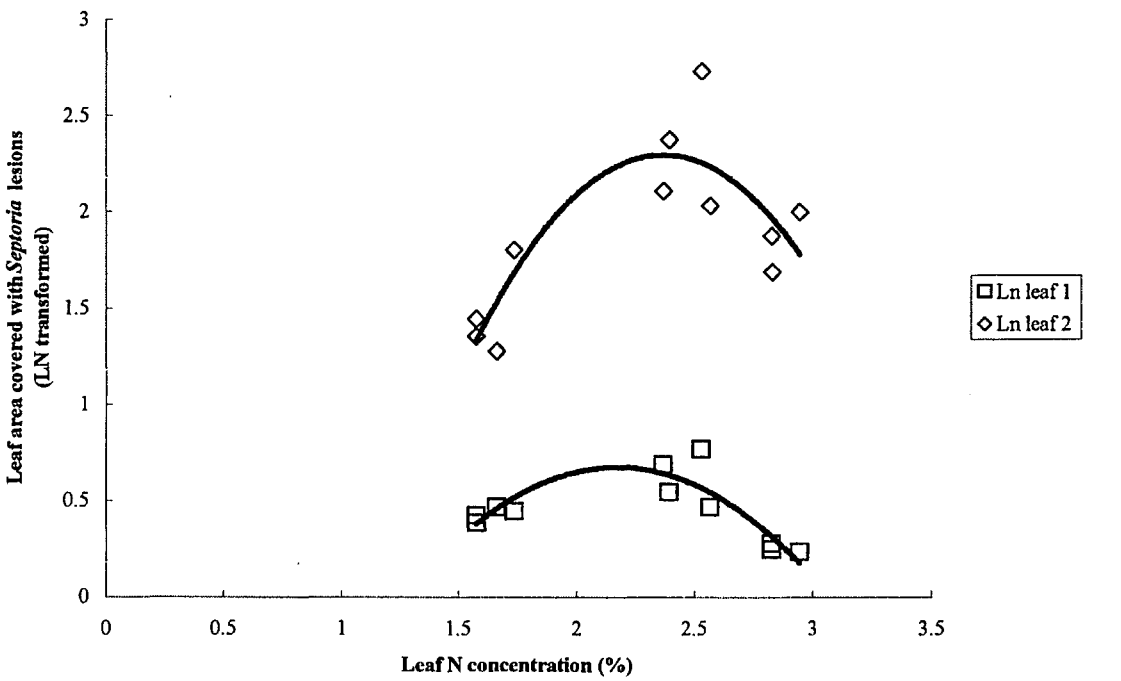


Figure 2.3.7 (b) The relationship between leaf N concentration and *Septoria* leaf area at 257 – 259 DAS (at approximately two weeks after anthesis) in Cycle I

Table 2.3.7 (a) Coefficients of quadratic regression analyses for relationships between leaf N concentration and *Septoria* leaf area (LN transformed) in Cycle I

DAS	*Leaf*	*a (t pr.)*	*b (t pr.)*	*Constant (t pr.)*	P *value*	R^2
241-246	*Leaf 1*	NS	NS	NS	= 0.275	-
(GS 65)		(= 0.200)	(= 0.185)	(= 0.099)		
	Leaf 2	-0.9	5.1	-5.9	= 0.001	0.76
		(= 0.031)	(= 0.020)	(= 0.024)		
	Leaf 3	NS	NS	NS	= 0.431	-
		(= 0.243)	(= 0.234)	(= 0.365)		
257-259	*Leaf 1*	-0.83	3.6	-3.2	= 0.004	0.69
		(= 0.001)	(= 0.002)	(= 0.004)		
	Leaf 2	-1.5	7.3	-6.4	= 0.006	0.65
		(= 0.011)	(= 0.008)	(= 0.021)		
271-273	*Leaf 1*	NS	NS	NS	= 0.226	-
		(= 0.374)	(= 0.444)	(= 0.889)		

In Cycle II, *Septoria* leaf area was negatively and linearly explained by leaf N concentration across the three varieties on leaf 3 ($P = 0.009$) at 168 – 172 days after sowing (i.e. after anthesis), on leaf 1 ($P = 0.009$) and leaf 2 ($P = 0.025$) at 186 – 193 days after sowing (i.e. at approximately three weeks after anthesis), although the R^2 was low (Fig. 2.3.7 (c), Table 2.3.7 (b)).

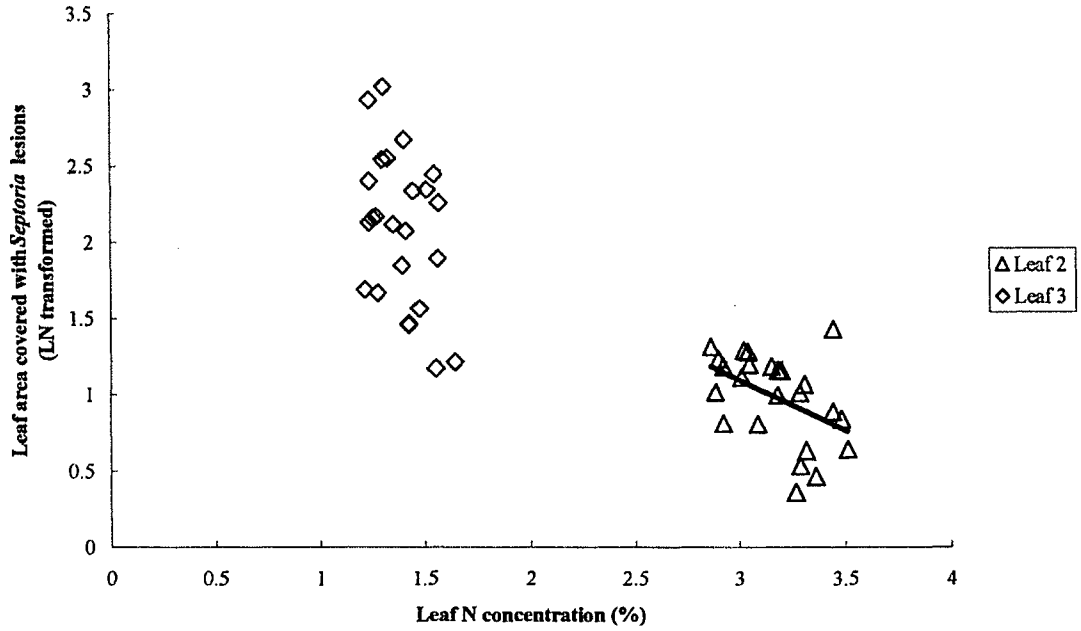


Figure 2.3.7 (c) The relationship between leaf N concentration and *Septoria* leaf area at 186 – 193 DAS (at approximately three weeks after anthesis) in Cycle II

Table 2.3.7 (b) Coefficients of linear regression analysis of the relationships between leaf N concentration and *Septoria* leaf area (LN transformed) in Cycle II

DAS	*Leaf*	*Coefficient (t pr.)*	*Constant (t pr.)*	P *value*	R^2
168-172 (GS 65)	*Leaf 1*	NS (= 0.714)	NS (= 0.815)	= 0.714	-
	Leaf 2	NS (= 0.233)	NS (= 0.193)	= 0.233	-
	Leaf 3	-2.0 (= 0.009)	5.6 (= 0.002)	= 0.009	0.24
186-193	*Leaf 1*	-0.77 (= 0.009)	3.1 (= 0.001)	= 0.009	0.24
	Leaf 2	-0.66 (= 0.025)	3.1 (= 0.002)	= 0.025	0.17
	Leaf 3	NS (= 0.053)	4.3 (< 0.001)	= 0.053	-

When only Hereward was considered, the relationship between leaf N concentration and *Septoria* leaf area was significant on leaf 2 (P = 0.043) at 168 – 172 days after sowing (i.e. after anthesis) and on leaf 3 (P = 0.050) at 186 – 193 days after sowing (i.e. at approximately three weeks after anthesis) with higher R^2 values (Fig. 2.3.7 (d), Table 2.3.7 (c)).

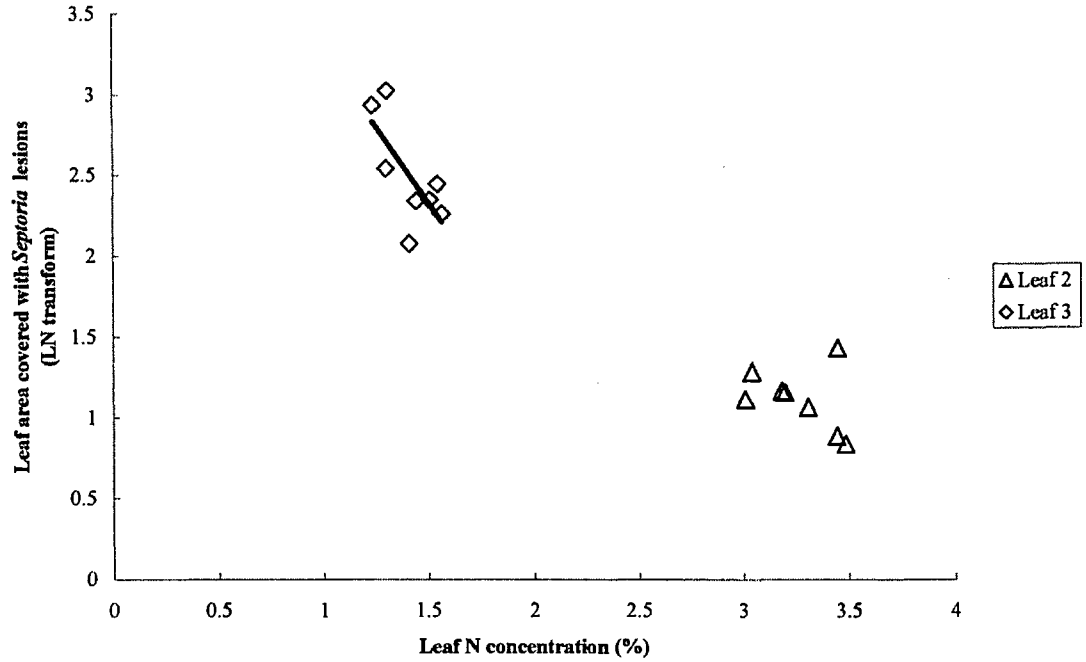


Figure 2.3.7 (d) The relationship between leaf N concentration and *Septoria* leaf area for Hereward at 186 – 193 DAS (at approximately three weeks after anthesis) in Cycle II

Table 2.3.7 (c) Coefficients of linear regression analysis of the relationships between leaf N concentration and *Septoria* leaf area (LN transformed) for Hereward in Cycle II

DAS	*Leaf*	*Coefficient (t pr.)*	*Constant (t pr.)*	P *value*	R^2
168-172 (GS 65)	*Leaf 1*	NS (= 0.840)	NS (= 0.950)	= 0.840	-
	Leaf 2	-1.6 (= 0.043)	7.2 (= 0.031)	= 0.043	0.44
	Leaf 3	NS (= 0.077)	4.9 (= 0.013)	= 0.077	-
186-193	*Leaf 1*	NS (= 0.688)	NS (= 0.449)	= 0.688	-
	Leaf 2	NS (= 0.385)	NS (= 0.123)	= 0.385	-
	Leaf 3	-1.9 (= 0.050)	5.2 (= 0.003)	= 0.050	0.42

In Cycle III-A, regression analysis was only significant on leaf 2 at 251 – 252 days after sowing (i.e. at heading) (P = 0.032), however, R^2 was high accounting for 65 % of the variance for this leaf (Fig. 2.3.7 (e), (f), Table 2.3.7 (d)).

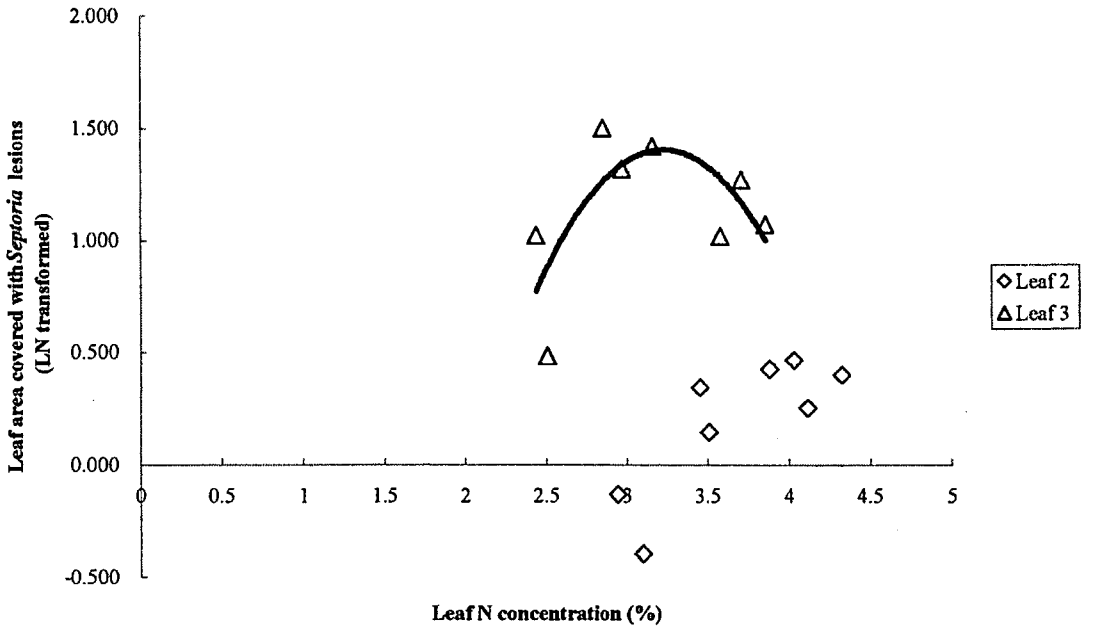


Figure 2.3.7 (e) The relationship between leaf N concentration (%) and *Septoria* leaf area at 251 – 252 DAS (at heading) in Cycle III-A

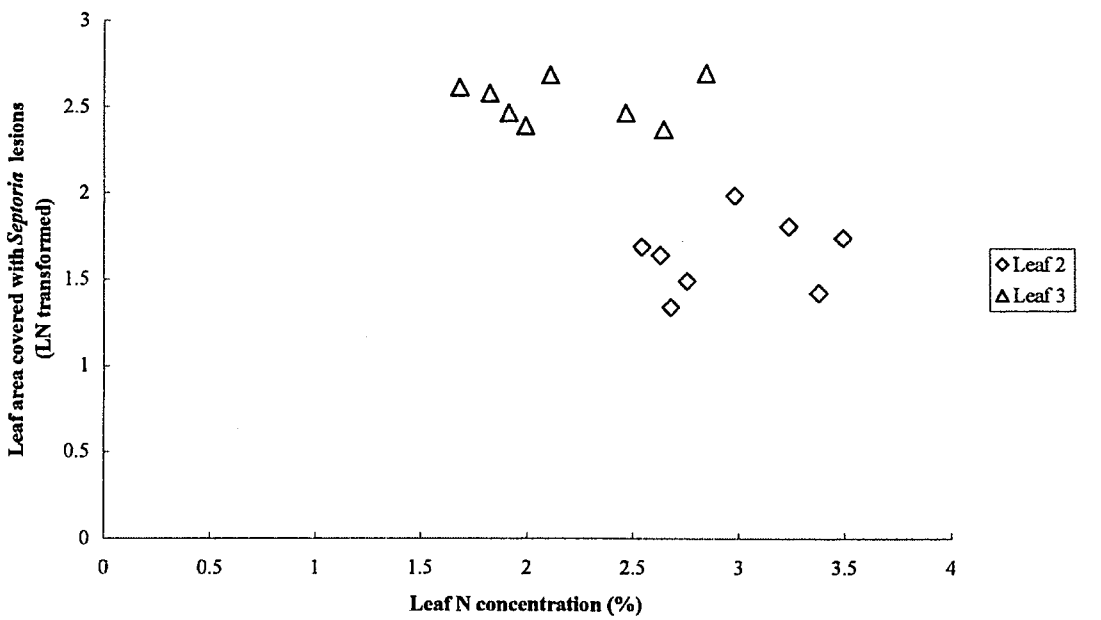


Figure 2.3.7 (f) The relationship between leaf N concentration and *Septoria* leaf area at 272 – 273 DAS (at approximately two weeks after heading) in Cycle III-A

Table 2.3.7 (d) Coefficients of quadratic regression analysis of the relationships between leaf N concentration and *Septoria* leaf area (LN transformed) in Cycle III-A

DAS	*Leaf*	*a (t pr.)*	*b (t pr.)*	*Constant (t pr.)*	P *value*	R^2
251-252	*Leaf 2*	NS	NS	NS	= 0.032	0.65
(GS 59)		(= 0.226)	(= 0.179)	(= 0.148)		
	Leaf 3	NS	NS	NS	= 0.173	-
		(= 0.093)	(= 0.087)	(= 0.112)		
272-273	*Leaf 2*	NS	NS	NS	= 0.764	-
		(= 0.587)	(= 0.576)	(= 0.670)		
	Leaf 3	NS	NS	NS	= 0.568	-
		(= 0.312)	(= 0.311)	(= 0.067)		

In Cycle III-B, *Septoria* leaf area was positively and linearly explained by leaf N concentration on leaf 3 (P = 0.033) and leaf 4 (P = 0.015) at 237 – 244 days after sowing (i.e. at approximately two weeks before anthesis) and on leaf 4 (P = 0.005) at 255 – 264 days after sowing (i.e. at anthesis) (Fig. 2.3.7 (g) and Table 2.3.7 (e)). The relationship was negative on leaf 1 (P = 0.019) at 255 – 264 days after sowing (i.e. at anthesis) (Fig. 2.3.7 (g), Table 2.3.7 (e)). On either leaf 1 or leaf 2 at 269 – 288 days after sowing (i.e. at approximately two weeks after anthesis), no significant relationship was observed between leaf N concentration and *Septoria* leaf (Fig. 2.3.7 (h), Table 2.3.7 (e)). When fitted for a quadratic curve, only leaf 1 (P = 0.045) and leaf 4 (P = 0.022) at 255 – 264 days after sowing (i.e. at anthesis) were significant and showed increased P values and similar R^2 values compared to those obtained with a linear regression (Fig. 2.3.7 (g), Table 2.3.7 (f)). Therefore, a linear regression was considered to be a better model in these cases.

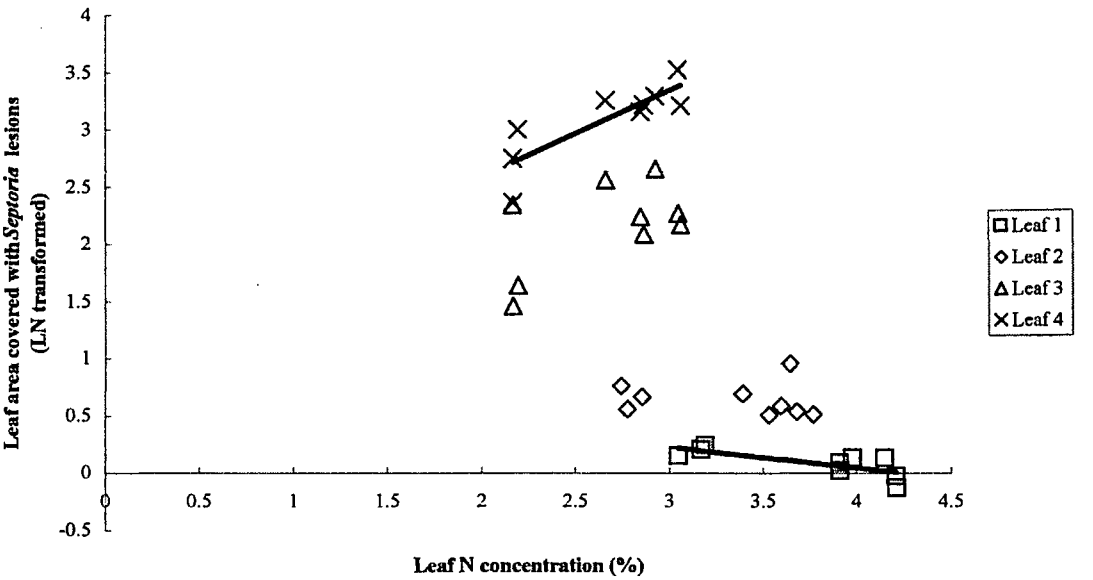


Figure 2.3.7 (g) The relationship between leaf N concentration and *Septoria* leaf area at 255 – 264 DAS (at anthesis) in Cycle III-B

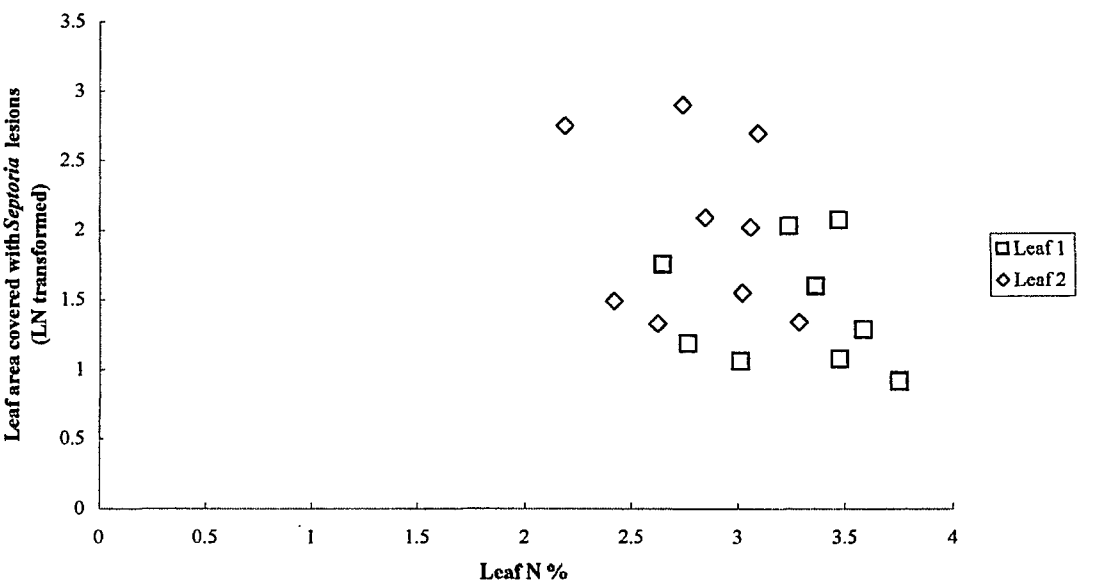


Figure 2.3.7 (h) The relationship between leaf N concentration and *Septoria* leaf area at 269 – 288 DAS (at approximately three weeks after anthesis) in Cycle III-B

Table 2.3.7 (e) Coefficients of linear regression analysis of the relationships between leaf N concentration and *Septoria* leaf area (LN transformed) in Cycle III-B

DAS	*Leaf*	*Coefficient (t pr.)*	*Constant (t pr.)*	P *value*	R^2
237-244	*Leaf 2*	NS (= 0.332)	NS (= 0.064)	= 0.332	-
(GS 49)					
	Leaf 3	0.41 (= 0.033)	NS (= 0.132)	= 0.033	0.43
	Leaf 4	0.67 (= 0.015)	NS (= 0.635)	= 0.015	0.54
255-264	*Leaf 1*	-0.18 (= 0.019)	0.78 (= 0.011)	= 0.019	0.51
(GS 65)					
	Leaf 2	NS (= 0.722)	NS (= 0.110)	= 0.722	-
	Leaf 3	NS (= 0.113)	NS (= 0.496)	= 0.113	-
	Leaf 4	0.75 (= 0.005)	NS (= 0.067)	= 0.005	0.66
269-288	*Leaf 1*	NS (= 0.660)	NS (= 0.183)	= 0.660	-
	Leaf 2	NS (= 0.554)	NS (= 0.133)	= 0.554	-

Table 2.3.7 (f) Coefficients of quadratic regression analysis of the relationships between leaf N concentration and *Septoria* leaf area (LN transformed) in Cycle III-B

DAS	*Leaf*	*a (t pr.)*	*b (t pr.)*	*Constant (t pr.)*	P *value*	R^2
237-244	*Leaf 2*	NS	NS	NS	= 0.637	-
(GS 49)		(= 0.854)	(= 0.831)	(= 0.751)		
	Leaf 3	NS	NS	NS	= 0.116	-
		(= 0.733)	(= 0.674)	(= 0.651)		
	Leaf 4	NS	NS	NS	= 0.062	-
		(= 0.747)	(= 0.675)	(= 0.723)		
255-264	*Leaf 1*	NS	NS	NS	= 0.045	0.53
(GS 65)		(= 0.293)	(= 0.332)	(= 0.393)		
	Leaf 2	NS	NS	NS	= 0.912	-
		(= 0.802)	(= 0.809)	(= 0.881)		
	Leaf 3	NS	NS	NS	= 0.180	-
		(= 0.308)	(= 0.283)	(= 0.333)		
	Leaf 4	NS	NS	NS	= 0.022	0.63
		(= 0.525)	(= 0.445)	(= 0.629)		
269-288	*Leaf 1*	NS	NS	NS	= 0.619	-
		(= 0.397)	(= 0.408)	(= 0.475)		
	Leaf 2	NS	NS	NS	= 0.851	-
		(= 0.989)	(= 0.963)	(= 0.841)		

2.3.8 Effects of *Septoria* diseases on yield and yield components

An attempt was made to explain grain yield from *Septoria* leaf area, however, no significant results were obtained (data not shown). The variability in TGW (presented in section 3.3.8.5) was significantly accounted for by *Septoria* leaf area (Fig. 2.3.8; Table 2.3.8). Grain Number per Area (GNA) could not be explained by the percentage of leaf area covered with *Septoria* lesions (data not shown).

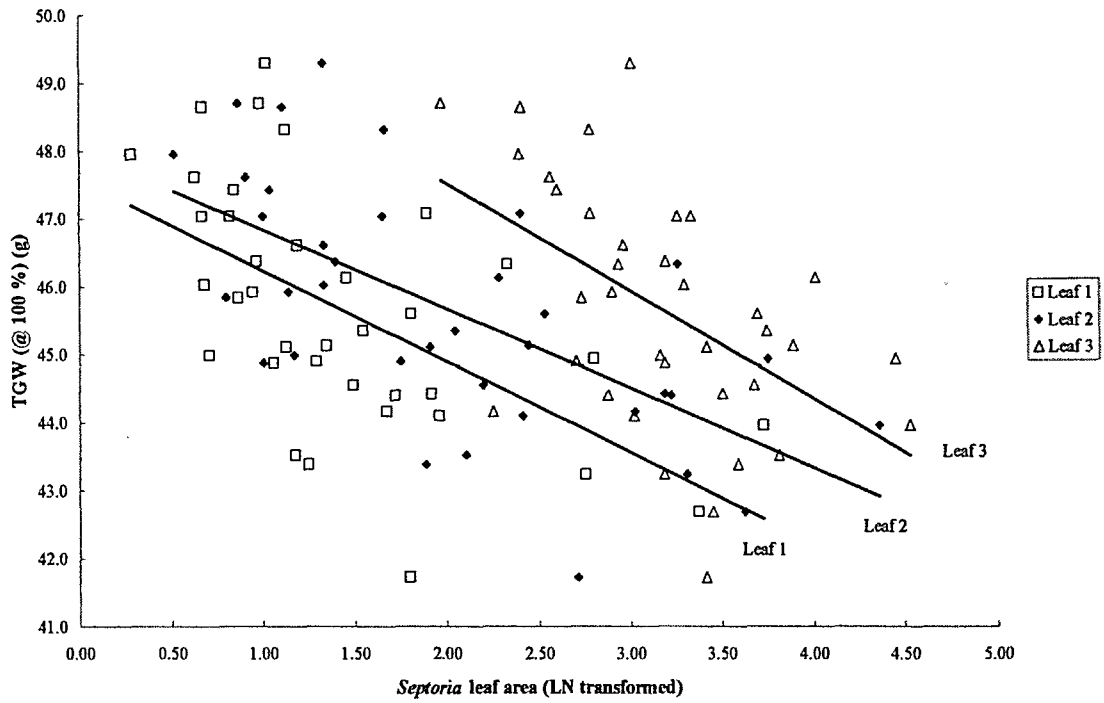


Figure 2.3.8 The relationship between *Septoria* leaf area on leaf 1, leaf 2 and leaf 3 at 269 – 288 DAS (at approximately three weeks after anthesis) and TGW at pre-harvest in Cycle III-B

Table 2.3.8 Coefficients of linear regression analysis of the relationship between leaf area covered with *Septoria* lesions (LN transformed) and TGW in Cycle III-B

Experiment	*Leaf*	*n*	*Coefficient (t pr.)*	*Constant (t pr.)*	P *value*	R^2
Cycle III-B	*Leaf 1*	36	-1.34 (< 0.001)	47.58 (< 0.001)	< 0.001	0.31
	Leaf 2	36	-1.17 (< 0.001)	48.01 (< 0.001)	< 0.001	0.38
	Leaf 3	36	-1.58 (= 0.002)	50.69 (< 0.001)	= 0.002	0.24

2.4 Discussion

2.4.1 Relationship between *Septoria* leaf area and senesced leaf area

The precision and accuracy of visual assessment of foliar diseases is questionable. Parker *et al.* (1995) argued that estimates of disease severity are usually more subjective compared to disease incidence, despite that disease severity needs to be directly estimated in many occasions especially where the relationship between incidence and severity is weak. In the study testing the reliability of visual estimates of disease severity on cereal leaves, Parker *et al.* (1995) reported that *S. tritici*, mildew and senescence were not estimated accurately or consistently by several experienced observers. In addition, distinguishing the boundary between disease necrosis and physiological senescence was mentioned to be a problem in assessing *S. tritici* (Parker *et al.*, 1995), which was the case in this study. In view of crop physiology, it is important to quantify the area of plants that are capable of photosynthesis. In this study, for example at anthesis, there was relatively a strong positive correlation between the leaf area covered with *Septoria* lesions and senesced leaf area, but as the time progressed, it became difficult to distinguish disease-originated necrosis and physiological senescence. The percentage of leaf area covered with *Septoria* lesions may account for, to some extent, yield variability, however, it is not likely to be an appropriate index when a greater leaf area is senesced without clear evidence that it is attributable to foliar diseases.

2.4.2 Interactions between treatments in severity of *Septoria* diseases

It is important to note that Cycle II was different from other field experiments in two respects. Firstly,

Cycle II was the only field experiment where variety was tested for the severity of *Septoria* diseases. Secondly, Cycle II was conducted in a year characterized with abnormal weather conditions compared to Cycle I and Cycle III. It is thus important to keep in mind the nature of Cycle II in interpreting the results. For Hereward a slightly better performance was observed for the plots treated with a mixture of epoxiconazole and trifloxystrobin in controlling *Septoria* diseases compared to untreated plots and the plots treated with a mixture of epoxiconazole and kresoxim-methyl, while there was no difference in severity of *Septoria* diseases between fungicide programmes for Malacca and Equinox. Hereward has been reported to be less responsive to strobilurin fungicides compared to other varieties such as Equinox (Bayles, 1999), but the results of this study might indicate a very complex mechanism of varieties in response to fungicides. As to the interactions between varieties and N rates, Equinox showed lower severity of *Septoria* diseases for the N rate of 130 kg ha^{-1} than that of 90 kg ha^{-1} on leaf 1 both at anthesis and at three weeks after anthesis, while there was no difference in the severity of *Septoria* diseases between N rates for Hereward and Malacca. It is not known why a lower N rate favoured *Septoria* diseases in Equinox, but it might be of some use to consider that Equinox is a feed variety and requires less amount of N compared to bread wheat varieties such as Hereward and Malacca. For example, the N rate of 130 kg ha^{-1} might have influenced the architecture of Equinox in such a way that assists escape from the spread of *Septoria* diseases to a greater extent than that of either Hereward or Malacca, which, of course, is a mere speculation and needs to be tested for confirmation.

2.4.3 Varieties and severity of *Septoria* diseases

Severity of *Septoria* diseases was tested only in Cycle II. Equinox showed the lowest severity of *Septoria*

diseases compared to Hereward and Malacca both at anthesis and at approximately three weeks after anthesis despite that its resistance both to *S. tritici* and *S. nodorum* has been scored the lowest in HGCA recommended lists of winter wheat (2002; 2003; 2004/2005), thus, expecting the severest *Septoria* diseases. As Cycle II was the only experiment where different varieties were tested in severity of *Septoria* diseases, it was not possible to investigate the degree as to such a difference in severity of *Septoria* diseases between varieties observed in this study would be attributable to the nature of varieties as well as that to environmental conditions.

2.4.4 Effects of Seed Rates on Severity of *Septoria* diseases

Overall the seed rate of 400 m^{-2} tended to show a greater *Septoria* leaf area than that of 100 m^{-2}. Considering that rainsplash mediates the spread of conidial inoculum, this result is not surprising. When the crop canopy is dense, more efficient spread of conidial inoculum would be expected. The hypothesis suggested by Lovell *et al.* (1997) that horizontal movement probably would amplify the effect of vertical movement of inoculum would likely to occur in a dense canopy rather than a scarce canopy.

2.4.5 Effects of fungicide programmes on severity of *Septoria* diseases

In Cycle I and Cycle III-B, *Septoria* leaf area was observed to be very often greater for untreated plots than the plots treated with fungicides, especially a mixture of epoxiconazole and trifloxystrobin indicating some meaning for applying fungicides to winter wheat with respect to the control of *Septoria* diseases. Among fungicide programmes, the plots treated with a mixture of epoxiconazole and trifloxystrobin seemed to have

performed the best with respect to the control of *Septoria* diseases. The interpretation of the results becomes more complex when Cycle II is included. At anthesis the plots treated with a mixture of epoxiconazole and trifloxystrobin tended to show a better performance as to the control of *Septoria* diseases compared to untreated plots and the plots treated with other fungicide programmes. The difference, however, disappeared by the time when a disease assessment was performed at approximately three weeks after anthesis. As to Cycle II, there seemed to have been no or very little benefit of applying fungicides to the crop whether it was attributable to the host factor such as shortened growing period of the crop or a relatively low pressure of *Septoria* diseases that might have been the case due to dry conditions during late spring and early summer in this year.

2.4.6 Effects of N on severity of *Septoria* diseases

In Cycle I, *Septoria* leaf area was the greatest for the plots treated with the N rate of 100 kg ha^{-1} compared to those treated with the N rate of either 0 kg ha^{-1} or 140 kg ha^{-1} at 257 – 259 days after sowing indicating that there might be an optimum N concentration for *Septoria* diseases to develop. As to Cycle II, *Septoria* leaf area was greater for the plots treated with the N rate of 90 kg ha^{-1} than that of 130 kg ha^{-1}, which agrees with Johnston *et al.* (1979) who observed an inverse linear relationship between N rate and *Septoria* blotch on spring wheat. More workers, however, found increased disease severity by *Septoria* diseases with increased N application rates (Prew *et al.*, 1983; Leitch and Jenkins, 1995). Neither Cycle III-A nor Cycle III-B did show any clear trend as to the relationship between N rates and *Septoria* leaf area. The relationship between leaf N concentration and the severity of *Septoria* diseases, therefore, was examined to further study the

effects of N on *Septoria* diseases. In Cycle I, the relationship between leaf N concentration and *Septoria* leaf area was well explained by a quadratic function indicating that there might be an optimum leaf N concentration for *Septoria* diseases to develop. In Cycle II, a negative linear relationship was observed between the two components. In Cycle I, the range of leaf N concentration on leaf 2 and leaf 3 fell on around 2 % and 3 % at anthesis, and at approximately two weeks after anthesis it fell to between 1.5 % and 3 % on leaf 1 and leaf 2. In Cycle II, the range of leaf N concentration on leaf 2 was around 3 – 3.5 % at approximately three weeks after anthesis, higher leaf N concentration compared to that in Cycle I considering that the measurement of leaf N concentration was conducted approximately a week later in Cycle II. From the relationship observed in Cycle I and Cycle II of this study between leaf N concentration and the severity of *Septoria* diseases, it is tempting to conclude that there might be an optimum range of leaf N concentration in absolute term for *Septoria* diseases to develop, for example, the severity of *Septoria* diseases showed a trend of decline in the range of leaf N concentration beyond 2.5 % both in Cycle I and Cycle II indicating this range of leaf N status is supra optimum for the development of *Septoria* diseases. Such a conclusion, however, is hard to draw. A trend of increase in the severity of *Septoria* diseases was observed between the range of leaf N concentration between 2.5 and 3 % both in Cycle III-A and Cycle III-B. Lower range of leaf N concentration is also debatable. In Cycle I, the lowest range of leaf N concentration observed was approximately 2 % at anthesis and 1.5 % around two weeks after anthesis. The severity of *Septoria* diseases in this range of leaf N concentration was relatively lower than that observed in higher range. In Cycle II, however, a trend of increase in the severity of *Septoria* diseases was observed, as the leaf N concentration declined between the range of 1 – 2 % of leaf N concentration. Supposing that there exists an optimum N

status of host plants for *Septoria* diseases, it is not likely to be of absolute nature. The optimum range of N status of host plants for *Septoria* diseases may be determined in the complex interactions not only with environmental factors but also with crop management practices, for example those affecting the crop canopy architecture such as seed rates.

2.4.7 Effects of *Septoria* diseases on yield and yield components

Being aware of the constraints of visual assessment, untreated plots showed a greater level of *Septoria* diseases than the plots treated with fungicide programmes both in Cycle I and Cycle III-B. The plots treated with a mixture of epoxiconazole and trifloxystrobin performed slightly better in controlling *Septoria* diseases than those treated with either epoxiconazole alone or with a mixture of epoxiconazole and kresoxim-methyl, however, it should be noted that these observations were made at a few occasions between a few weeks interval and therefore, could not quantify *Septoria* diseases that were present during the whole growing season. In Cycle II the severity of *Septoria* diseases was hardly affected by fungicide programmes. *Septoria* diseases are known to reduce TGW (Simon *et al.*, 2002), which was observed in this study. At the same time, grain yield at pre-harvest could not be explained by the severity of *Septoria* diseases measured at a few given occasions during grain filling period. One should, however, be aware that relating the severity of *Septoria* diseases of such pin-point measurements to final grain yield hardly reflects the impact of the diseases on yield, as the influence of *Septoria* diseases occurs in an accumulated manner in time scale. In this study it was not possible to determine the area under the disease progress curve for the small number of observations. Despite that the greater variability in grain yield is usually attributable to Grain Number per

Area (GNA) as it will be discussed in the next chapter, a significant reduction in TGW would obviously damage grain yield. Therefore, a certain level of crop protection measure is needed to ensure production of acceptable yield.

Chapter 3

Growth and Dry Matter Accumulation

3.1 Introduction

3.1.1 Leaf Area Index (LAI) and Leaf Area Duration (LAD)

Leaf Area Index (LAI) is the term that describes the sum of the area of all leaves per unit of ground and Green Leaf Area Duration (GLAD) is calculated as the integral of green LAI over time (Loomis and Connor, 1992). During early phase of crop growth following emergence, light interception linearly increases with increasing LAI but then stops increasing when mutual shading of leaves starts to occur. GLAD has been known to often show a close relationship with yield irrespective of different growth conditions due to climate, agronomic practices and varieties, however the closeness of the relationship between GLAD and yield seems to be reduced under conditions where LAI reaches a high value to the extent that excess LAI does not contribute to light interception (Evans *et al.*, 1975). GLAD might be a convenient and useful concept in order to estimate the productivity of a canopy but its limitation due to lack of sufficient physiological justification in cases where GLAD poorly reflects the real productivity of LAI should be recognized.

3.1.2 Canopy Light Interception and Dry Matter Accumulation

Monsi and Saeki (1953) calculated light intensity at a given depth of a plant canopy using a function of cumulative LAI and an extinction coefficient. The concept has been very often applied to the domain of

crop science as well as ecology to obtain quantitative understanding of canopy light interception and dry matter accumulation. Monsi and Saeki (1953) also suggested the possible existence of optimal LAI that gives maximum photosynthetic efficiency as a plant canopy, while the presence of asymptotic plateau in the relationship between crop growth rate and LAI was proposed by other workers (Brougham, 1956; McCree and Troughton, 1966). Yoshida (1972) argued that dry matter production is the balance between photosynthesis and respiration and therefore favoured the concept of asymptotic plateau to that of optimal LAI as well as denied the presence of any pronounced optimum LAI in dry matter production by a crop community except for some cases, for example where the leaf angle of a canopy is reduced at high LAI values. A linear relationship has been well established between absorbed photosynthetic active radiation (PAR) and total dry matter production (Monteith, 1977; Gallagher and Biscoe, 1978). Gallagher and Biscoe (1978) discussed that a greater total dry matter is produced by a particular variety or treatment through either increased radiation absorption or improved radiation conversion into dry matter or both. They, however, noted that an increased total dry matter production does not always mean an increased yield, because fractions between the two do vary. It is through the increase in this fraction termed as harvest index (HI) rather than in total dry matter accumulation that yield has increased with modern varieties of wheat (Austin *et al.*, 1980; Loomis and Connor, 1992). Increased biomass production by modern varieties in comparison with older varieties was, however, reported by Austin *et al.* (1989).

3.1.3 Radiation Use Efficiency (RUE)

Sinclair and Muchow (1999) recognized Monteith (1977) to be the first to establish both experimental and

theoretical grounds for the relationship between intercepted solar radiation and accumulated crop dry matter. Monteith (1977) obtained through experimental results the value of approximately 1.4 g of crop mass accumulated per MJ of intercepted solar radiation for various crops such as apples and sugar beet grown without apparent stress. Other workers followed his study. Gallagher and Biscoe (1978) obtained RUE of 3.0 g MJ^{-1} PAR with wheat and barley. With rice, Horie and Sakuratani (1985) measured total crop dry weight including roots and absorbed PAR from transplanting until 20 days after heading and estimated RUE of 2.88 g MJ^{-1} PAR. It was 2.75 g MJ^{-1} PAR when they estimated for the period from transplanting until 40 days after heading. They argued that the capacity of rice plants for dry matter production decreases in the latter part of grain filling period due to the reduced sink capacity. RUE is considered to be constant in non-stressed environment (Monteith, 1977) and underlying assumption is that dry matter loss due to maintenance respiration and growth respiration is proportional to total gross photosynthesis (Charles-Edwards, 1982). RUE is expected to be lowered under stressed conditions (Biscoe and Gallagher, 1978; Sinclair and Muchow, 1999).

3.1.4 Yield and Yield Components

As has been already mentioned in section 3.1.3, fractions of dry matter accumulated in grains are important in understanding yield formation. In the case of cereals such as wheat, yield is the product of the number of grains per unit area and mean individual grain mass (Green, 1984). Most of the time grain yield is strongly associated with number of grains per unit area (Spiertz and Ellen, 1978; Ellen and Spiertz, 1980; Fischer, 1985; Abbate *et al.*, 1995; Bindraban, 1997) and therefore understanding variation in number of grains per

unit area is the key to explain yield variability (Slafer and Savin, 1994; Bindraban *et al.*, 1998). The number of grains per unit area is the product of the number of ears per unit area, the number of spikelets per ear and the number of florets per spikelet. It is thus particularly important to be aware when and how these components are formed in the life cycle of wheat. The number of spikelets per ear and the number of florets per spikelet are determined during the reproductive phase. In wheat the early sign of inflorescence initiation can be recognized as the double ridge (Kirby, 1993) followed by a sequential differentiation of bracts, spikelets and florets up to around the time when the flag leaf has fully emerged (Kirby, 1988). Important yield determining components such as grain sets and grain size are known to be under the influence of the position of spikelets within a given inflorescence as well as that of florets within a spikelet (Rawson and Evans, 1970; Bremner, 1972). Superior grains are characterized by earlier setting and relatively good access to vascular systems transferring assimilates during grain filling, while inferior ones tend to set later and have limited access to assimilate supply (Evans *et al.*, 1972; Cook and Evans, 1978). Compensatory mechanisms are very often found between yield components (Evans and Wardlaw, 1976). Earlier-determined components are compensated for by later-determined ones (Evans and Wardlaw, 1976).

3.1.5 Carbohydrate Accumulation in Grains

Cell number in endosperm increases mainly at the outermost layer until as late as 18 days after fertilization (Hoshikawa, 1961). Brocklehurst (1977) studied the effects of removal of all florets except the basal floret of the four central spikelets at anthesis and 15 days after anthesis on grain weight of wheat. Floret removal at anthesis aimed to provide an excess of assimilate available to the remaining grains during the period of cell

division in the endosperm, while that at 15 days after anthesis gave an excess of assimilate during the time when development was due entirely to cell expansion in the endosperm. Reducing floret number at anthesis increased the number of endosperm cells and individual grain weight was increased to a much greater extent when florets were reduced at anthesis than 15 days after anthesis. A suggestion was made that cell number in the endosperm was a major factor controlling the rate of increase in dry matter as well as final dry weight. Close positive correlations between endosperm cell number and final grain dry weight have been observed with wheat by other workers as well (Singh and Jenner, 1982; Singh and Jenner, 1984). Recognizing the importance of endosperm cell number in final grain dry weight, the next question to follow would be which factor regulates endosperm cell number. Singh and Jenner (1982) tested, in experiments conducted in a controlled environment, the hypothesis that endosperm cell number is regulated by the substrate available within the endosperm for cell division and growth, however, they observed no association between substrate concentration and endosperm cell number. In the following experiments investigating the association between the availability of nutrients within the endosperm and endosperm cell number, Singh and Jenner (1984) recognized that cellular division was responsive to nutritional supply, yet observed no evidence that cellular division was regulated directly by the nutritive factors in concentration-dependent manner. Carbohydrate accumulation in grains is affected by environmental factors (Brocklehurst *et al.*, 1978; Caley *et al.*, 1990). A number of observations have been made in the field as well as phytotron experiments that the duration of grain filling increases when temperature falls (Evans and Wardlaw, 1976; Bindraban, 1997). A reduction in grain size is observed as the main effect of high temperature during grain filling period (Jenner, 1994) due to a reduction in the grain filling period which was not compensated for by the increased rate of

dry matter accumulation (Sofield *et al.*, 1977).

3.1.6 Sink-Source Relationship

In earlier stages of crop research, attention was particularly paid to canopy formation in the early stages of crop development as well as source factors to produce and provide assimilates. It was with the recognition of yield being limited by not only source factors but also sink factors that the potential sink size has become an important area of investigation (Evans and Wardlaw, 1976). The three components mentioned above, however, are equally significant and are interrelated to one another in forming yields (Evans and Wardlaw, 1976). Evans *et al.* (1975) argued that under most circumstances, CO_2 assimilation after anthesis contributes to as high as 90 – 95 % of the carbohydrate in grain of wheat. Exclusive contribution of the flag leaf in supplying assimilates to ears has been reported for wheat (Wardlaw, 1965; Rawson and Hofstra, 1969). On the other hand, substantial contributions of pre-anthesis assimilate for wheat and barley as high as 43 % were reported by Gallagher *et al.* (1975). Bidinger *et al.* (1977), however, criticized that the subtraction of dry matter weight of non-grain parts at maturity from that at anthesis, the method of estimating the pre-anthesis contribution employed by Gallagher *et al.* (1975) was subjected to errors of overestimating the pre-anthesis contribution by ignoring the dry matter weight lost during the grain filling period. It is well established that under stressed conditions during grain filling, the contribution of reserve assimilates becomes greater (Rawson and Evans, 1971; Gallagher and Biscoe, 1978a; Austin *et al.*, 1980a). In a number of studies source/sink ratio was artificially altered by means of shading, defoliation and removal of florets to elucidate physiological mechanisms controlling grain-filling and yield of cereals (Wardlaw *et al.*, 1965;

Jenner, 1980; Borghi *et al.*, 1986; Takahashi *et al.*, 1994; Takahashi and Kanazawa, 1996). Source reduction by methods such as defoliation and shading before anthesis tends to reduce grain number per area, while that after anthesis reduces both grain number per area and individual grain mass (Savin and Slafer, 1991). Inconsistent effects of source/sink alteration, however, were often found (Slafer and Savin, 1994). Sink reduction by halving and degraining spikes is often followed by an increase in the remaining grains (Radley and Thorne, 1981), although there are cases where no or little increase of grain weight was observed (Martinez-Carrasco and Thorne, 1979).

3.1.7 Effects of Fungicides on Dry Matter Accumulation

For a number of fungicides, the consequence of application to a field-grown wheat crop is very often recognized as prolonged leaf area duration (Jones, 1998; Saunders and Salmon, 2000; Jones *et al.*, 2001) whether it is disease-related or physiology-related in its cause. The application of fungicides therefore could be interpreted as the relative expansion of source compared to sink. Davies *et al.* (1984) observed a prolonged photosynthetic activity per unit area of flag leaf at 40, 54 and 61 days after flag leaf emergence in response to propiconazole application at GS 39 with no observed effect of fungicide application on either grain number or mean grain weight.

They suspected that the delay in senescence was too late to contribute to the grain yield. In field experiments carried out under various sites, seasons and environments to investigate the effects of late-season applications of propiconazole and tridemorph fungicides on grain yield and breadmaking quality, Gooding *et*

al. (1994) observed a longer green leaf area duration and an increased rate of grain filling following the application of propiconazole (125 g a.i.) plus tridemorph (250 g a.i.) at GS 39 and GS 59. Grain maturation was delayed by the fungicide application. When considering all the field experiments conducted in this study, grain yield was increased by the fungicide application by 7.6 % among which 5.5 % was attributable to the increase in TGW.

As to strobilurin fungicides, Gooding *et al.* (2000) reported that azoxystrobin treated at or close to flag leaf emergence and ear emergence prolonged green flag leaf area duration (GFLAD) and increased mean grain mass. The degree of prolongation of GFLAD as well as that of the increase in mean grain mass was greater compared to the effects brought about by triazole and morpholines (Dimmock and Gooding, 2002a). Ruske *et al.* (2001), in one-year field experiment where the effect of adding azoxystrobin to a triazole fungicide programme was tested on four wheat varieties (i.e. Hereward, Malacca, Charger and Consort), observed delayed senescence of the flag leaf following the application of azoxystrobin at flag leaf emergence only at stem extension and flag leaf emergence, and at flag leaf and ear emergence by 7 days, 9 days and 14 days respectively compared to the control (i.e. triazole only), with no variety and fungicide interaction being observed. A week of delayed senescence of the flag leaf was followed by, on average, an increase in grain yield by 0.7 t ha^{-1}, while the yield of Hereward remained the same. They hypothesized that the grains of Hereward had reached the maximum size by the time the delayed senescence became effective.

Variable grain size in response to partial degraining among 20 wheat varieties was documented by Ma *et al.*

(1990) indicating that some varieties may become sink-limited more easily than others, although environmental factors play an important role. A more detailed study focusing on the change in grain weight during the grain filling period in relation to leaf area index would be required to elucidate the sink-source relationship of strobilurin-treated wheat crops.

3.1.8 Effects of N on Dry Matter Accumulation, Yield and Yield Components

In experiments where spring wheat plants were grown in perlite-contained containers and were supplied with solutions of either low or high N concentration at three different development stages (i.e. (1) from double ridges to floral initiation, (2) from floret initiation to ear emergence, and (3) from ear emergence to maturity), Langer and Liew (1973) observed that the number of spikelets per ear could be increased by N supply only at or close to the double ridge stage. As to the number of florets per spike, which is affected to a much greater extent than the number of spikelets per ear, they reported that the supply of high N in the period between double ridges to floral initiation gave a mean increase of 1.60 grains per spikelet, while that between floret initiation to ear emergence gave 0.85 grains per spikelet and the extra N supply after ear emergence had no significant effect. Such differences in grain number per spikelet due to the different timings of high N application were reflected in grain weight per ear, where more than doubled grain weight, an increase of about 35 %, and no effect were observed respectively for the application between double ridges to floret initiation, from floret initiation to ear emergence, and after ear emergence. In a field experiment on winter wheat, Spiertz and Ellen (1978), however, observed no increase in the number of spikelets per ear by raising the early dressing in the middle of March from 50 to 100 kg ha^{-1} and argued that it was due to the adequate

supply from soil N.

Not as great as the influence of grain number per area in most of the cases, yield variations are, however, partly attributable to variations in grain mass (Darwinkel, 1983). Fertilizer N application of high rates sometimes results in a reduction in individual grain mass (McCabe and Gallagher, 1993). In addition to the rates of fertilizer N, its type is also important. Despite that urea has been reported to be an inferior N source to ammonium nitrate on a wide range of crops by a number of workers (Chaney and Paulson, 1988 and references quoted therein), urea could be useful depending on environmental conditions, for example, when soil condition becomes dry in summer and prevents N uptake from roots (Barraclough and Haynes, 1996). In the UK, late foliar urea application is a well-established practice for increasing protein content of bread-making wheat crops (Pushman and Bingham, 1976; Gooding *et al.*, 1991). No consistent increase or decrease in yield in response to foliar N applied at pre- and post-flowering over years and locations was reported by Woolfolk *et al.* (2002), however, in many cases no or little yield increases were observed following the foliar N application after heading (Finney *et al.*, 1957; Powlson *et al.*, 1987). Similar observation of lack of yield response to urea N applied around anthesis was reported by Below *et al.* (1985) for maize.

3.1.9 Objectives and Hypothesis of Chapter 3

This chapter deals with the Objective (II) in the section of '*1.2 Aim, Objectives and Approach*' of Chapter 1 (page 6), i.e., "to understand the effects of the use of strobilurin fungicides in regard of fertilizer N rates on

growth, canopy size, dry matter accumulation, partitioning, yield components and yield of winter wheat".

Firstly this chapter will test the hypothesis that there is no synergistic effect between the use of strobilurin fungicides and fertilizer N rates on growth, canopy size, dry matter accumulation, partitioning, yield components and yield. Secondly it will test the hypothesis that the use of a strobilurin fungicide in a disease control programme in wheat does not affect growth, canopy size, dry matter accumulation, partitioning, yield components and yield.

Four field experiments under a factorial design of fungicide programmes and N rates as factors were performed. The fungicide programmes included the use of the triazole epoxiconazole alone, and in mixture with either kresoxim-methyl or trifloxystrobin, which are strobilurins. A range of N rates were used which varied according to the specific requirements for each field experiment. Variety was added as an extra factor in two of the four field experiments.

3.2 Materials and Methods

3.2.1 General

The data sets from Cycle I, Cycle II, Cycle III-B and Cycle III-C were used in this chapter. Sampling dates are given in Appendix 3. After foliar disease assessment described in Chapter 2, a part of the samples was taken as 'Sub-sample'. Sub-sampled plants were separated into different plant parts. The method of taking sub-samples and that of separating sub-samples into different plant parts is explained in 3.2.2 and 3.2.3 respectively. The data sets of the percentage of senesced leaf area was transformed in the same way as that of *Septoria* diseases described in Chapter 2 (Eq. 2.1).

3.2.2 Sub-Samples

1) Cycle I

All the sampled plants were taken as a sub-sample for this field experiment.

2) Cycle II

Sampled plants were categorized into effective tillers type 1 (i.e. the tillers that boar fully emerged inflorescence), effective tillers type 2 (i.e. the tillers that boar inflorescence and were not categorized into type 1), ineffective tillers (i.e. the tillers that did not bear inflorescence), green leaves and senesced leaves. The number was counted for each category. Firstly, 40 effective tillers of type 1 were taken as a sub-sample from the main samples and the ratio of sub-sampled tillers to main sampled tillers in number was calculated.

Secondly, effective tillers of type 2 were taken as a sub-sample in the similar ratio to effective tillers of type 1. The sub-sample of type 1 and that of type 2 were mixed.

3) Cycle III-B

Sampled plants were categorized into effective tillers type 1 (i.e. the tillers that bore fully emerged inflorescence), effective tillers type 2 (i.e. the tillers that bore inflorescence and were not categorized into type 1), ineffective tillers (i.e. the tillers that did not bear inflorescence), green leaves and senesced leaves. The number was counted for each category and its fresh weight measured. Approximately 20 % of the main sample in fresh weight was taken as a sub-sample for each category. The sub-samples of each category were mixed together.

4) Cycle III-C

Fresh weight of the sampled plants was weighed for each plot. Approximately 20 % of the main samples in fresh weight were taken as a sub-sample.

3.2.3 Separation of Sub-Samples into Plant Parts

1) Cycle I and Cycle III-B

Sub-sampled plants were separated into stem and leaf at samplings before heading. At samplings after heading, sub-sampled plants were separated into stem, leaf and spike (Photograph 6 and 7). Leaves were further separated based on their position and their colour. Details are given in Appendix 5. Spikes were

further separated into rachis/chaff and grain for some of the sub-samples in Cycle III-B.

2) Cycle II

Sub-sampled plants were separated into upper stem, lower stem, upper leaf, lower leaf and spike. Upper stem was defined as the stem beyond the middle point between auricles of leaf 2 and leaf 3, while lower stem was defined as the stem below the middle point between auricles of leaf 2 and leaf 3. Leaf 1 and leaf 2 were defined as upper leaf and leaves below leaf 2 were defined as lower leaf (Appendix 5). Spikes were further separated into rachis/chaff and grain for some of the sub-samples.

3.2.4 Calculation of Green Leaf Area Duration (GLAD) in Cycle III-B

LAIs measured at six samplings were linearly interpolated and the area under the LAI line between the two consecutive sampling dates was determined as the area of either a rectangle (S1, S2, S3, S4) or a triangle (S5) (Fig. 3.2.4). GLAD (days) was calculated as the sum of S1, S2, S3, S4 and S5.

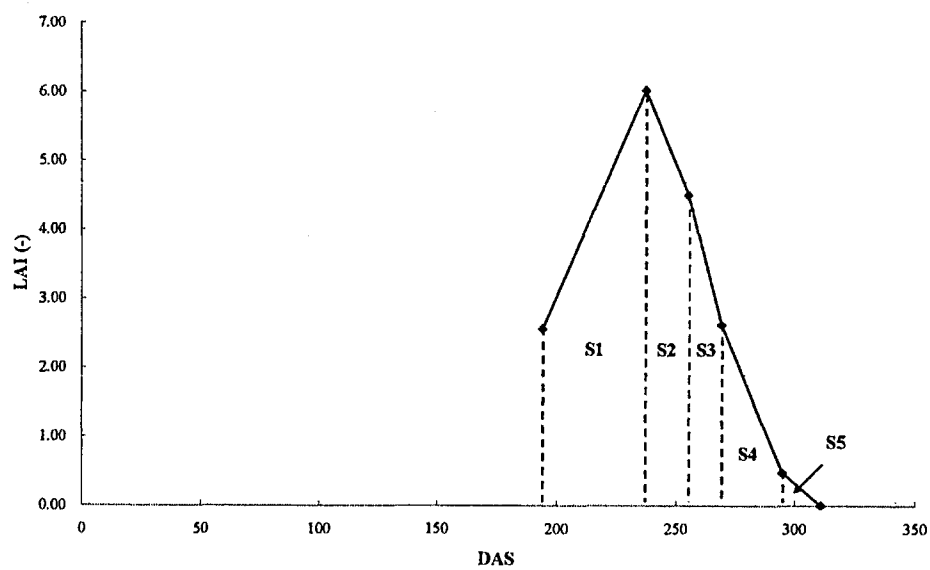


Figure 3.2.4 Visual presentation of the calculation method of GLAD in Cycle III-B

3.2.5 Photosynthetic Active Radiation (PAR) absorbed by crop canopy

Daily absorbed PAR by a given crop canopy (La) was calculated from daily LAI and daily solar radiation (L0) for cycle III-B during the period from GS 39 until harvest. Extinction coefficient (K) and reflection coefficient (Pc) were set at 0.44 (Dreccer, 1999) and 0.08 (Lövenstein *et al.*, 1995) respectively. Daily LAI was linearly interpolated from five measurements taken between the period from GS 39 until harvest. The fraction of PAR (400 to 700 nm) was assumed to be 50 % of solar radiation (Loomis and Connor, 1992; Dreccer, 1999). Regression analysis was performed between calculated PAR absorption and dry matter accumulation during the period from GS 39 until harvest.

$$La = (I\text{-}Pc) \times 0.5 \times L0 \times (1\text{-}\exp^{-K \times LAId})$$

Pc: 0.08 (Lövenstein *et al.*, 1995)

K: 0.44 (Dreccer, 1999)

3.3 Results

3.3.1 Senesced Leaf Area

The results of Cycle II and Cycle III-B are presented in this section. There was no interaction in senesced leaf area between fungicide programmes and N rates in Cycle II and Cycle III-B.

Fungicide Programmes

In Cycle II, fungicide programmes did not cause significant differences in percentage of senesced leaf area except on leaf 3 observed at 168 – 172 days after sowing (i.e. after anthesis) where the plots treated with a mixture of epoxiconazole and trifloxystrobin showed a smaller percentage of senesced leaf area than untreated plots and those treated with epoxiconazole alone by 3.06 % and 1.70 % respectively ($P = 0.022$) (Table 3.3.1 (a)).

Table 3.3.1 (a) The percentage of senesced leaf area (LN transformed) for fungicide programmes in Cycle II

DAS	*Leaf*	*untrt*	*epoxi*	*epoxi + kresoxim*	*epoxi + triflo*	*L.S.D.*	*P value*	*CV %*
168-172	*Leaf 2*	0.42	0.45	0.47	0.47	NS	= 0.983	102.4
(GS 65)		(1.48)	(1.31)	(1.53)	(1.48)			
	Leaf 3	1.58	1.50	1.34	1.13	0.30	= 0.022	32.6
		(6.65)	(5.29)	(4.82)	(3.59)			
186-193	*Leaf 1*	0.94	0.93	0.92	0.89	NS	= 0.981	43.2
		(2.49)	(2.75)	(2.32)	(2.48)			
	Leaf 2	1.5	1.2	1.5	1.5	NS	= 0.627	54.4
		(5.7)	(4.6)	(5.6)	(5.7)			
	Leaf 3	3.3	3.0	3.3	3.1	NS	= 0.334	17.7
		(31.5)	(25.1)	(28.9)	(24.3)			

untrt: untreated; epoxi: epoxiconazole; kreso: kresoxim-methyl; triflo: trifloxystrobin

In Cycle III-B the percentage of senesced leaf area was affected differently by fungicide programmes for any measurement made after GS 65. At 255 – 264 days after sowing (i.e. at anthesis), the plots treated with epoxiconazole alone showed a smaller percentage of senesced leaf area than untreated plots and those treated with a mixture of epoxiconazole and trifloxystrobin by 0.54 % and 0.24 % respectively and the plots treated with a mixture of epoxiconazole and trifloxystrobin showed a smaller percentage of senesced leaf area than untreated plots by 0.30 % on leaf 2 ($P < 0.001$) (Table 3.3.1 (b)). On leaf 3, untreated plots showed a greater percentage of senesced leaf area than the plots treated with epoxiconazole alone and those treated with a mixture of epoxiconazole and trifloxystrobin by 9.17 % and 6.70 % respectively ($P = 0.002$) (Table 3.3.1 (b)). On leaf 4, the plots treated with a mixture of epoxiconazole and trifloxystrobin showed a smaller percentage of senesced leaf area than untreated plots by 13.14 % ($P = 0.041$) (Table 3.3.1 (b)).

At 269 – 288 days after sowing (i.e. at approximately three weeks after anthesis), both on leaf 1 and leaf 2, the plots treated with a mixture of epoxiconazole and trifloxystrobin showed a smaller percentage of senesced leaf area than untreated plots and those treated with epoxiconazole alone by 10.90 % and 2.01 % respectively on leaf 1 and by 25.40 % and 5.81 % respectively on leaf 2 and the plots treated with epoxiconazole alone showed a smaller percentage of senesced leaf area than untreated plots by 8.89 % on leaf 1 and by 19.59 % on leaf 2 ($P < 0.001$) (Table 3.3.1 (b)). On leaf 3, untreated plots showed a greater percentage of senesced leaf area than the plots treated with epoxiconazole alone and those treated with a mixture of epoxiconazole and trifloxystrobin by 21.69 % and 37.59 % respectively and the plots treated with epoxiconazole alone showed a greater percentage of senesced leaf area than those treated with a mixture of epoxiconazole and trifloxystrobin by 15.9 % ($P < 0.001$) (Table 3.3.1 (b)).

Table 3.3.1 (b) The percentage of senesced leaf area (LN transformed) for fungicide programmes in Cycle III-B

DAS	*Leaf*	*untrt*	*epoxi*	*epoxi + triflo*	*L.S.D.*	*P value*	*CV %*
237-244	*Leaf 3*	0.75	0.72	0.56	NS	= 0.261	45.1
(GS 49)		(1.91)	(1.88)	(1.48)			
	Leaf 4	2.15	2.22	2.09	NS	= 0.721	17.9
		(9.37)	(9.29)	(9.63)			
255-264	*Leaf 2*	0.89	0.66	0.77	0.11	< 0.001	16.7
(GS 65)		(2.13)	(1.59)	(1.83)			
	Leaf 3	2.72	2.04	2.19	0.36	= 0.001	18.3
		(17.00)	(7.83)	(10.30)			
	Leaf 4	4.21	4.13	3.96	0.20	= 0.041	5.8
		(68.20)	(62.56)	(55.06)			
269-288	*Leaf 1*	2.13	1.57	1.18	0.39	< 0.001	28.6
		(14.02)	(5.13)	(3.12)			
	Leaf 2	3.03	2.21	1.67	0.37	< 0.001	19.0
		(31.52)	(11.93)	(6.12)			
	Leaf 3	4.19	3.77	3.32	0.24	< 0.001	7.3
		(71.54)	(49.85)	(33.95)			

untrt: untreated; epoxi: epoxiconazole; triflo: trifloxystrobin

3.3.2 Leaf Area Index (LAI)

Cycle I

In Cycle I, LAI was measured only at heading. No interaction between fungicide programmes and N rates was observed for LAI of any leaf layer observed in this field experiment. LAI was greater for the plots treated with the N rate of 140 kg ha^{-1} than those treated with that of 100 kg ha^{-1} by 0.5 (P = 0.039) (Table 3.3.2 (a)). The same was observed for LAI of flag leaf with the difference being 0.24 (P = 0.019) (Table 3.3.2 (a)). There was no difference both in LAI and that of flag leaf between fungicide programmes. The

plots treated with a mixture of epoxiconazole and trifloxystrobin showed a greater LAI of non-flag leaf compared to those treated with epoxiconazole alone by 0.52 (P = 0.036), while no difference was observed between the two N rates (Table 3.3.2 (a)).

Table 3.3.2 (a) LAI at heading (GS 59) in Cycle I

Fungicide Programmes	*N rates (kg ha^{-1})*	*LAI*	*LAI of flag leaf*	*LAI of non-flag leaf*
epoxi	-	5.6	1.89	3.72
epoxi + kreso	-	5.7	1.81	3.91
epoxi + triflo	-	6.2	1.94	4.23
-	*100*	5.6	1.76	3.83
-	*140*	6.1	2.00	4.08
P value	*Fungicide Programmes*	= 0.113	= 0.491	= 0.036
	N rates	= 0.039	= 0.019	= 0.097
L.S.D.		0.5 (N rates)	0.20 (N rates)	0.38 (Fungicide)
CV %		7.7	9.9	7.4

epoxi: epoxiconazole; kreso: kresoxim-methyl; triflo: trifloxystrobin

Cycle II

LAI observed in Cycle II was generally very low due to late sowing and the lack of N for leaf formation (Photograph 4). There was no interaction in LAI between fungicide programmes and N rates in Cycle II. Fungicide programmes did not affect LAI in any different manner (Table 3.3.2 (b)). As to N rates, the plots treated with the N rate of 130 kg ha^{-1} always showed a greater LAI than those treated with that of 90 kg ha^{-1} except for that of upper leaf at anthesis. The difference in LAI between the two N rates was 0.18 for total leaf (P = 0.010), 0.12 for lower leaf (P = 0.002) respectively at 168 – 172 days after sowing (i.e. after anthesis). It was 0.22 for total leaf (P = 0.001), 0.09 for upper leaf (P = 0.038) and 0.13 for lower leaf (P <

0.001) respectively at 186 – 193 days after sowing (Table 3.3.2 (c)) (i.e. at approximately three weeks after anthesis).

Table 3.3.2 (b) LAI for fungicide programmes in Cycle II

DAS	*LAI*	*untrt*	*epoxi*	*epoxi + kresoxim*	*epoxi + triflo*	*P value*	*CV %*
168-172 (GS 65)	*Total*	1.88	1.85	1.96	1.94	= 0.582	14.8
	Upper	1.12	1.07	1.12	1.11	= 0.719	13.7
	Lower	0.75	0.77	0.84	0.83	= 0.227	18.8
186-193	*Total*	1.41	1.53	1.56	1.49	= 0.422	18.2
	Upper	1.06	1.11	1.12	1.08	= 0.726	16.1
	Lower	0.35	0.43	0.43	0.41	= 0.173	29.8

untrt: untreated; epoxi: epoxiconazole; kreso: kresoxim-methyl; triflo: trifloxystrobin

Table 3.3.2 (c) LAI for N rates in Cycle II

DAS	*LAI*	*90 kg N*	*130 kg N*	*L.S.D.*	*P value*	*CV %*
168-172 (GS 65)	*Total*	1.82	2.00	0.13	= 0.010	14.8
	Upper	1.08	1.14	NS	= 0.089	13.7
	Lower	0.74	0.86	0.07	= 0.002	18.8
186-193	*Total*	1.39	1.61	0.13	= 0.001	18.2
	Upper	1.05	1.14	0.08	= 0.038	16.1
	Lower	0.34	0.47	0.06	< 0.001	29.8

kg N: kg N ha^{-1}

Cycle III-B

The change in LAI in Cycle III-B is shown both for fungicide programmes and N rates as means over fungicide programmes and N rates respectively (Fig. 3.3.2 (a), (b)). No interaction was observed in LAI between fungicide programmes and N rates. There was no difference in LAI between fungicide

programmes before and at flag leaf emergence.

After the onset of leaf senescence, significant difference started being observed in LAI between untreated plots and those treated with a mixture of epoxiconazole and trifloxystrobin and continued until 295 – 302 days after sowing (i.e. at approximately six weeks after anthesis). At 255 – 264 days after sowing (i.e. at anthesis), the plots treated with a mixture of epoxiconazole and trifloxystrobin showed a greater LAI than untreated plots by 0.56 ($P = 0.003$) (Table 3.3.2 (d)). At 269 – 288 days after sowing (i.e. at approximately three weeks after sowing), the plots treated with a mixture of epoxiconazole and trifloxystrobin showed a greater LAI than untreated plots and those treated with epoxiconazole alone by 0.98 and 0.63 respectively and the plots treated with epoxiconazole alone showed a greater LAI than untreated plots by 0.35 ($P < 0.001$) (Table 3.3.2 (d)). At 295 – 302 days after sowing (i.e. at approximately six weeks after sowing), the plots treated with a mixture of epoxiconazole and trifloxystrobin showed a greater LAI than untreated plots and those treated with epoxiconazole alone by 0.62 and 0.37 respectively and the plots treated with epoxiconazole alone showed a greater LAI than untreated plots by 0.25 ($P < 0.001$) (Table 3.3.2 (d)).

With respect to N rates, at 194 – 198 days after sowing, the plots that received no N fertilizer showed a smaller LAI than the plots treated with the N rate of 100 kg ha^{-1} and 140 kg ha^{-1} by 0.95 and 0.99 respectively ($P < 0.001$) (Table 3.3.2 (d)). At 237 – 244 days after sowing (i.e. at approximately two weeks before anthesis) ($P < 0.001$) and 255 – 264 days after sowing (i.e. at anthesis) ($P < 0.001$), the greater the N rate, the greater the LAI (Table 3.3.2 (d)). At 269 – 288 days after sowing (i.e. at approximately three

weeks after anthesis), the plots that received no N fertilizer showed a smaller LAI than the plots treated with the N rate of 100 kg ha^{-1} and 140 kg ha^{-1} by 0.90 and 1.17 respectively ($P < 0.001$) (Table 3.3.2 (d)). At 295 – 302 days after sowing (i.e. at approximately six weeks after anthesis), the plots treated with the N rate of 140 kg ha^{-1} showed a greater LAI than the plots treated with the N rate of 0 kg ha^{-1} and 100 kg ha^{-1} by 0.40 and 0.24 respectively ($P = 0.003$) (Table 3.3.2 (d)). LAI of separate leaf layers is presented in Appendix 6.

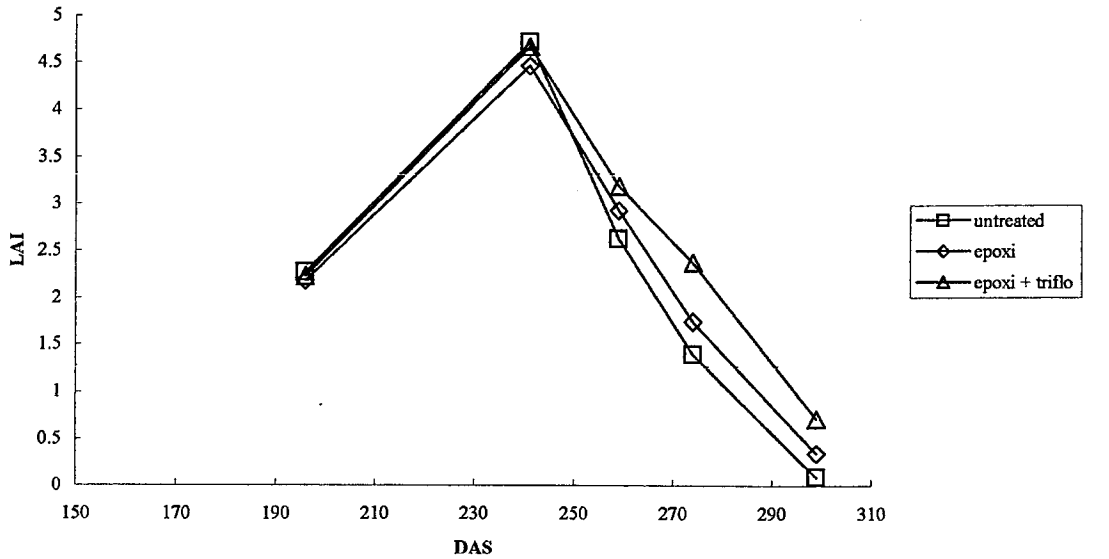


Figure 3.3.2 (a) Change in LAI for fungicide programmes in Cycle III-B

epoxi: epoxiconazole; triflo: trifloxystrobin

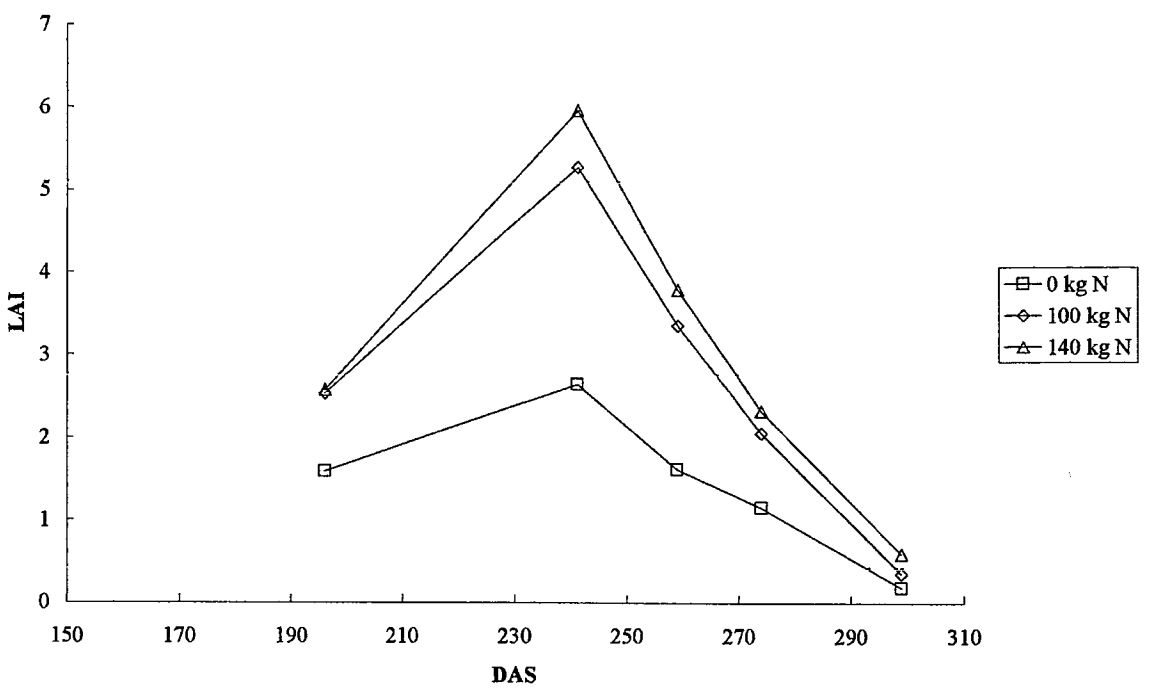


Figure 3.3.2 (b) Change in LAI for N rates in Cycle III-B

kg N: kg N ha^{-1}

Table 3.3.2 (d) LAI in Cycle III-B

Fungicide Programmes	*N rates (kg ha^{-1})*	*DAS*				
		194-198	237-244 (GS 49)	255-264 (GS 65)	269-288	295-302
untrt	-	2.27	4.72	2.63	1.40	0.09
epoxi	-	2.18	4.47	2.93	1.75	0.34
epoxi + triflo	-	2.23	4.67	3.19	2.38	0.71
-	*0*	1.58	2.64	1.61	1.15	0.19
-	*100*	2.53	5.27	3.35	2.05	0.35
-	*140*	2.57	5.96	3.79	2.32	0.59
P value	*Fungicide*	= 0.826	= 0.549	= 0.003	< 0.001	< 0.001
	N rates	< 0.001	< 0.001	< 0.001	< 0.001	= 0.003
	Interaction	= 0.709	= 0.877	= 0.295	= 0.890	= 0.098
L.S.D.	*Fungicide*	NS	NS	0.30	0.33	0.21
	N rates	0.31	0.50	0.30	0.33	0.21
CV %		16.3	12.8	12.0	21.6	66.0

untrt: untreated; epoxi: epoxiconazole; triflo: trifloxystrobin

3.3.3 Green Leaf Area Duration (GLAD) in Cycle III-B

In Cycle III-B, GLAD was calculated from the first sampling (194 – 198 DAS) to the sixth sampling (311 – 323 DAS) that was conducted within a week before harvest (see section 3.2.4). There was no interaction in GLAD between fungicide programmes and N rates. GLAD was significantly greater for the plots treated with a mixture of epoxiconazole and trifloxystrobin than for untreated plots and those treated with epoxiconazole alone by 32 days (Table 3.3.3 (a)). The greater the N rate, the greater the GLAD ($P < 0.001$) (Table 3.3.3 (a)).

Table 3.3.3 (a) GLAD from GS 32 to maturity in Cycle III-B (days)

Fungicide	*untrt*	*epoxi*	*epoxi + triflo*	*P value*	*L.S.D.*	*CV %*
	280	288	320	= 0.029	30	12.2

N rates (kg ha^{-1})	*0*	*100*	*140*	*P value*	*L.S.D.*
	176	336	376	< 0.001	30

untrt: untreated; epoxi: epoxiconazole; triflo: trifloxystrobin

Polynomial equation (Eq.3.3) was tested to explain aboveground DMW as well as grain yield from GLAD (Fig. 3.3.3). Coefficients and constant were significant at 0.1 % probability level for aboveground DMW (Table 3.3.3 (b)). As to grain yield, coefficients were significant at 0.1 % probability level but not constant (Table 3.3.3 (b)). 87 % of aboveground DM and 88 % of grain yield were accounted for by GLAD (Table 3.3.3 (b)).

$$Y = a X^2 + b X + c \qquad \text{(Eq. 3.3)}$$

Y: Aboveground DMW or grain yield ($\times 10^3$ kg ha^{-1})

X: GLAD

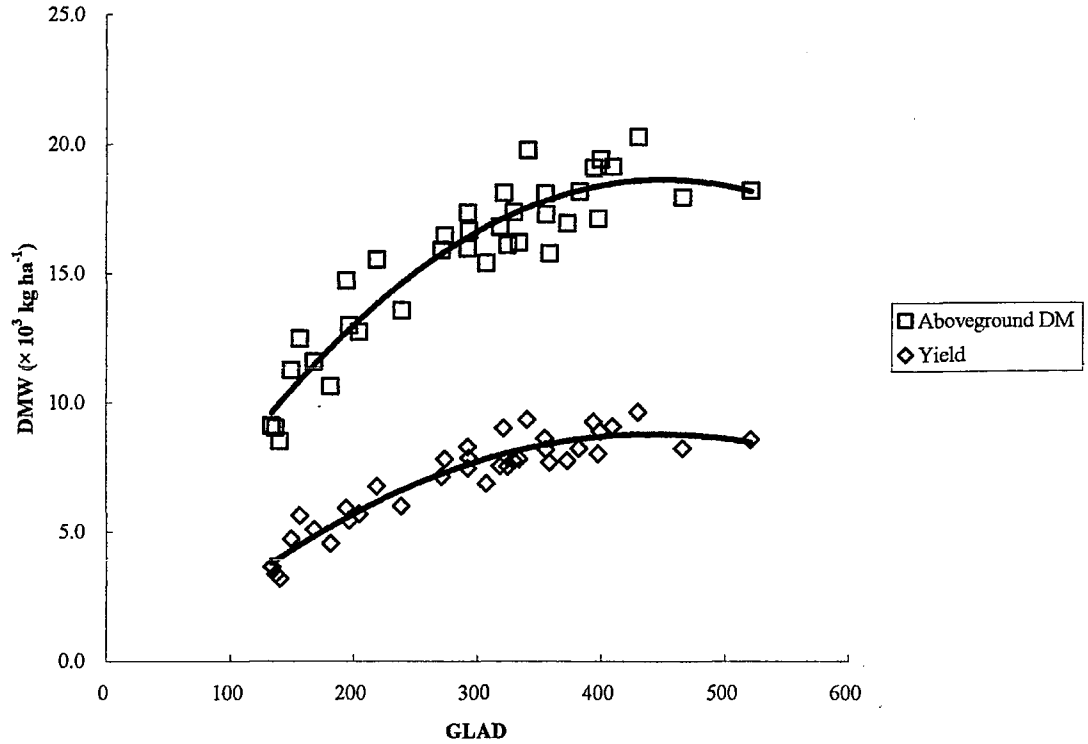


Figure 3.3.3 The relationships between GLAD and aboveground DMW as well as grain yield at pre-harvest in Cycle III-B

Table 3.3.3 (b) Coefficients of polynomial regression analysis of the relationships between GLAD and aboveground DMW as well as grain yield

Response Variable	*a (t pr.)*	*b (t pr.)*	*Constant (t pr.)*	*P value*	R^2
Aboveground DMW	-9.0×10^{-5}	8.13×10^{-2}	0.36	< 0.001	0.87
(× 10^3 kg ha^{-1})	(< 0.001)	(< 0.001)	(= 0.808)		
Grain Yield	-5.2×10^{-5}	4.64×10^{-2}	-1.48	< 0.001	0.88
(× 10^3 kg ha^{-1})	(< 0.001)	(< 0.001)	(= 0.066)		

3.3.4 PAR interception by crop canopy

PAR interception was calculated by the method described in the section of 3.2.5 of *'Materials and Methods'*. There was no interaction in PAR interception between fungicide programmes and N rates. The plots treated with a mixture of epoxiconazole and trifloxystrobin showed a greater PAR interception than untreated plots and those treated with epoxiconazole alone by 67 MJ and 44 MJ respectively ($P < 0.001$) (Table 3.3.4). The plots that received no fertilizer N showed a smaller PAR interception than those treated with the N rate of 100 kg ha^{-1} and 140 kg ha^{-1} by 165 MJ and 197 MJ respectively and the plots treated with the N rate of 100 kg ha^{-1} showed a smaller PAR interception than those treated with that of 140 kg ha^{-1} by 32 MJ ($P < 0.001$) (Table 3.3.4).

Table 3.3.4 PAR intercepted by crop canopies from GS 32 to maturity in Cycle III-B (MJ)

Fungicide	*untrt*	*epoxi*	*epoxi + triflo*	*P value*	*L.S.D.*	*CV %*
Programmes	479	502	546	< 0.001	30	7.0
N rate (kg ha^{-1})	***0***	***100***	***140***	***P value***	***L.S.D.***	
	388	553	585	< 0.001	30	

untrt: untreated; epoxi: epoxiconazole; triflo: trifloxystrobin

3.3.5 Aboveground Dry Matter Weight

Interactions

There was no interaction in aboveground DMW between fungicide programmes and N rates in Cycle I. In Cycle II an interaction in aboveground DMW between fungicide programmes and N rates was observed at

168 – 172 days after sowing (i.e. after anthesis) where the plots treated with a mixture of epoxiconazole and kresoxim-methyl showed a different response to the two N rates. The plots treated with the N rate of 130 kg ha^{-1} showed a greater aboveground DMW than those treated with that of 90 kg ha^{-1} by 1000 kg ha^{-1} for the plots treated with a mixture of epoxiconazole and kresoxim-methyl but not for untreated plots and those treated with other fungicide programmes. With the N rate of 90 kg ha^{-1} the plots treated with a mixture of epoxiconazole and kresoxim-methyl showed a greater aboveground DMW than untreated plots and those treated with other fungicide programmes with the difference ranging from 600 to 800 kg ha^{-1} ($P = 0.036$) (Fig. 3.3.5 (a)).

In Cycle III-B an interaction in aboveground DMW was observed between fungicide programmes and N rates at 295 – 302 days after sowing (i.e. at approximately six weeks after anthesis) where the three fungicide treatments including untreated affected aboveground DMW differently in response to the three N rates. The plots treated with the N rate of 140 kg ha^{-1} showed a greater aboveground DMW than those treated with that of 100 kg ha^{-1} by 2000 kg ha^{-1} for the plots treated with a mixture of epoxiconazole and trifloxystrobin, while there was no difference in aboveground DMW between the two N rates for untreated plots and those treated with epoxiconazole alone ($P = 0.002$) (Fig. 3.3.5 (b)). With no N application, untreated plots showed a greater aboveground DMW than those treated with epoxiconazole alone and those treated with a mixture of epoxiconazole and trifloxystrobin by 3100 kg ha^{-1} and 1600 kg ha^{-1} respectively and the plots treated with epoxiconazole alone showed a smaller aboveground DMW than those treated with a mixture of epoxiconazole and trifloxystrobin by 1500 kg ha^{-1}, while with the N application rate of 100 kg ha^{-1}, untreated

plots showed a smaller aboveground DMW than those treated with epoxiconazole alone and those treated with a mixture of epoxiconazole and trifloxystrobin by 2300 kg ha^{-1} and 1400 kg ha^{-1} respectively and with the N application rate of 140 kg ha^{-1}, the plots treated with a mixture of epoxiconazole and trifloxystrobin showed a greater aboveground DMW than untreated plots and those treated with epoxiconazole alone by 2500 kg ha^{-1} and 1800 kg ha^{-1} respectively ($P = 0.002$) (Fig. 3.3.5 (b)). It appeared that the interaction was mainly caused by the yield reduction following the application of fungicides whether epoxiconazole alone or a mixture of epoxiconazole and trifloxystrobin where no N was applied.

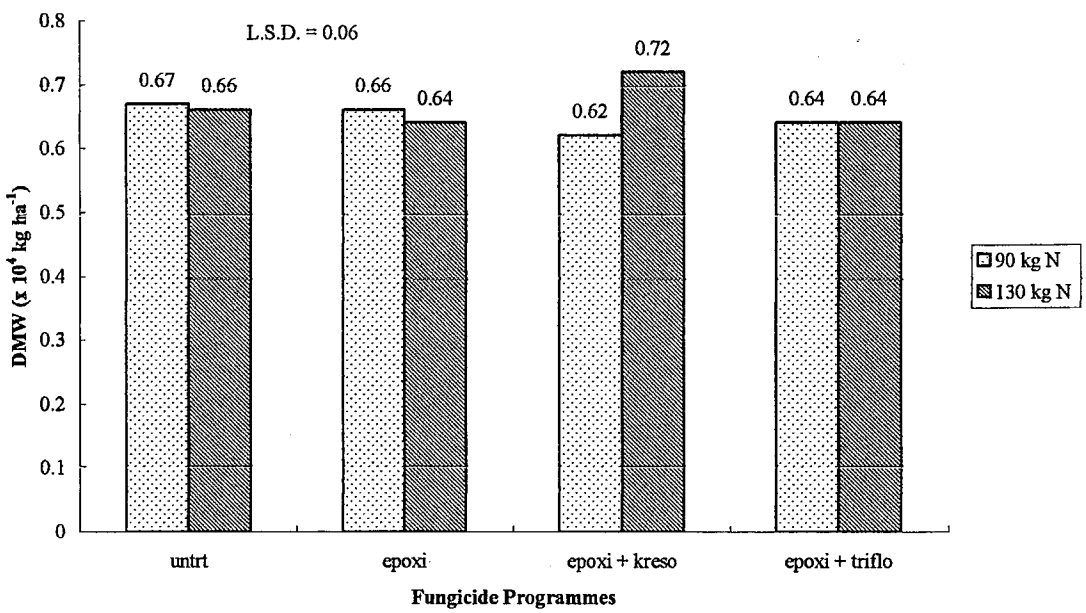


Figure 3.3.5 (a) The interaction in aboveground DMW between fungicide programmes and N rates at 168 – 172 DAS (after anthesis) in Cycle II

untrt: untreated; epoxi: epoxiconazole; kreso: kresoxim-methyl; triflo: trifloxystrobin

kg N: kg N ha^{-1}

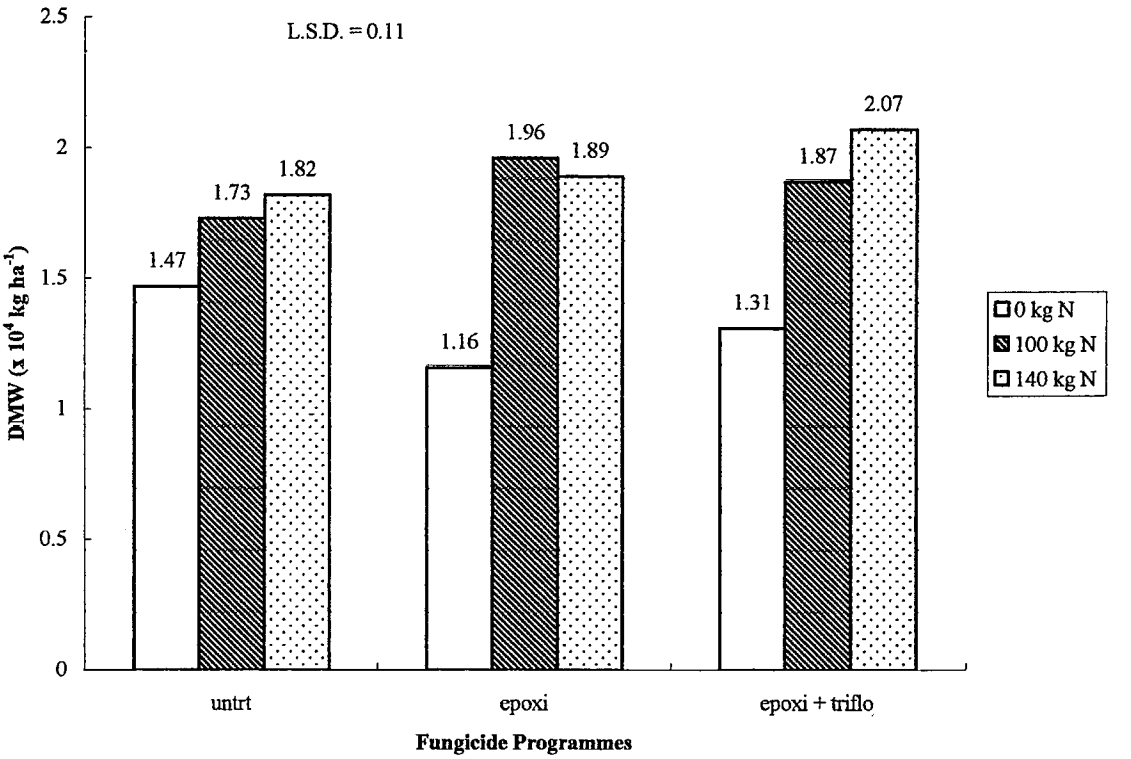


Figure 3.3.5 (b) The interaction in aboveground DMW between fungicide programmes and N rates at 295 – 302 DAS (approximately six weeks after anthesis) in Cycle III-B

untrt: untreated; epoxi: epoxiconazole; triflo: trifloxystrobin

kg N: kg N ha^{-1}

Fungicide Programmes

No difference in aboveground DMW was observed with pre-harvest samples between fungicide programmes in Cycle I, Cycle II and Cycle III-B. In Cycle III-C untreated plots showed a smaller aboveground DMW than those treated with fungicides at pre-harvest ($P < 0.001$) (Table 3.3.5 (a)). The difference between untreated plots and those treated with fungicides ranged from 2500 kg ha^{-1} to 3400 kg ha^{-1}. No difference in aboveground DMW was observed at other samplings in any of the field experiments (data not shown).

Table 3.3.5 (a) Aboveground DMW at pre-harvest sampling for fungicide programmes ($\times 10^4$ kg ha^{-1})

Fungicide Programmes	*Cycle I*	*Cycle II*	*Cycle III-B*	*Cycle III-C*
untrt	1.59	1.15	1.54	1.74
epoxi	1.68	1.16	1.55	1.99
epoxi + kreso	1.65	1.14	-	2.00
epoxi + triflo	1.75	1.14	1.60	2.08
P value	= 0.095	= 0.946	= 0.527	< 0.001
L.S.D.	NS	NS	NS	0.10
CV %	7.5	12.0	9.4	6.9

untrt: untreated; epoxi: epoxiconazole; kreso: kresoxim-methyl; triflo: trifloxystrobin

N rates

Both in Cycle I and Cycle III-B, the plots that received no N fertilizer showed a smaller aboveground DMW at pre-harvest than those treated with the N rate of 100 kg ha^{-1} and 140 kg ha^{-1} by 680 kg ha^{-1} and 690 kg ha^{-1} respectively in Cycle I ($P < 0.001$) and by 580 kg ha^{-1} and 550 kg ha^{-1} respectively in Cycle III-B ($P < 0.001$) (Table 3.3.5 (b)). In Cycle II the plots treated with the N rate of 130 kg ha^{-1} showed a greater aboveground DMW at pre-harvest than those treated with that of 90 kg ha^{-1} by 70 kg ha^{-1} ($P = 0.041$) (Table 3.3.5 (b)). In Cycle III-C there was no difference in aboveground DMW at pre-harvest between the N rates of 100 kg ha^{-1} and 200 kg ha^{-1} (Table 3.3.5 (b)).

Looking at the time change in aboveground DMW, in Cycle I the plots that received no N fertilizer always showed a smaller aboveground DMW than those given N fertilizer (Fig. 3.3.5 (c)). The difference in aboveground DMW between the N rates of 100 kg ha^{-1} and 140 kg ha^{-1} was significant before anthesis. At 214 – 216 days after sowing (i.e. at approximately four weeks before anthesis) and 229 – 232 days after

sowing (i.e. at approximately two weeks before anthesis), the plots treated with the N rate of 100 kg ha^{-1} showed a greater aboveground DMW than those treated with that of 140 kg ha^{-1} by 450 kg ha^{-1} ($P < 0.001$) and by 900 kg ha^{-1} ($P < 0.001$) respectively (Fig. 3.3.5 (c)). In Cycle II the difference in aboveground DMW between the N rates of 90 kg ha^{-1} and 130 kg ha^{-1} was not significant until pre-harvest (Fig. 3.3.5 (d)). In Cycle III-B the plots that received no N always showed a smaller aboveground DMW than those given N fertilizer (Fig. 3.3.5 (e)). The difference in aboveground DMW between the N rates of 100 kg ha^{-1} and 140 kg ha^{-1} was significant only at 255 – 264 days after sowing (i.e. at anthesis) when the former showed a smaller aboveground DMW than the latter by 800 kg ha^{-1} ($P < 0.001$) (Fig. 3.3.5 (e)).

Table 3.3.5 (b) Aboveground DMW at pre-harvest sampling for N rates (× 10^4 kg ha^{-1})

N rates (kg ha^{-1})	*Cycle I*	*Cycle II*	*Cycle III-B*	*Cycle III-C*
0	1.21	-	1.18	-
90	-	1.11	-	-
100	1.89	-	1.76	1.92
130	-	1.18.	-	-
140	1.90	-	1.73	-
200	-	-	-	1.98
P value	< 0.001	= 0.041	< 0.001	= 0.091
L.S.D.	0.11	0.07	0.12	NS
CV %	7.5	12.0	9.4	6.9

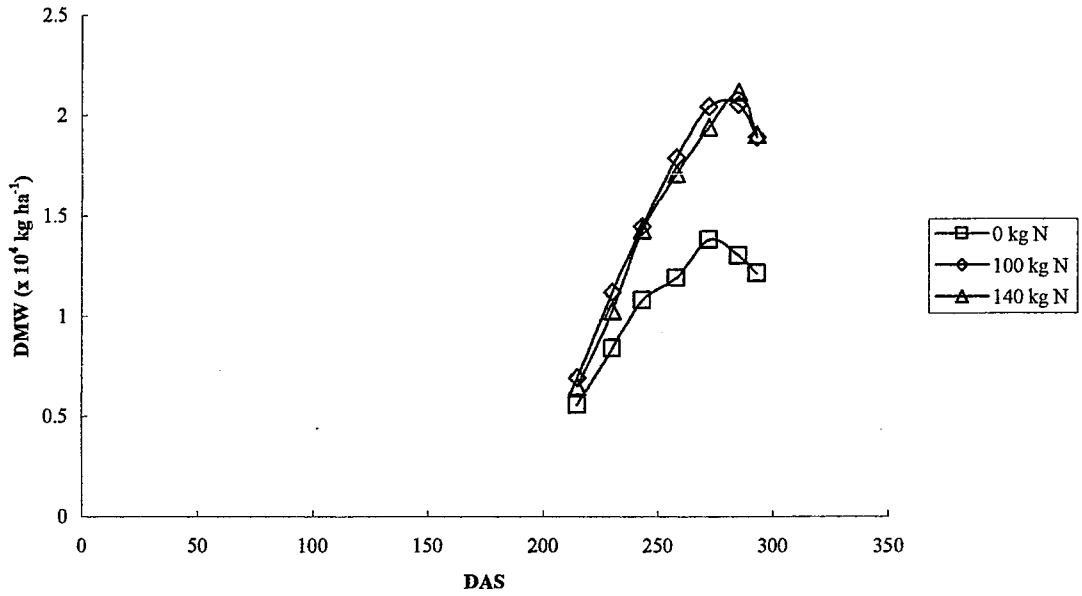


Figure 3.3.5 (c) The time change in aboveground DMW for N rates in Cycle I

kg N: kg ha^{-1} N

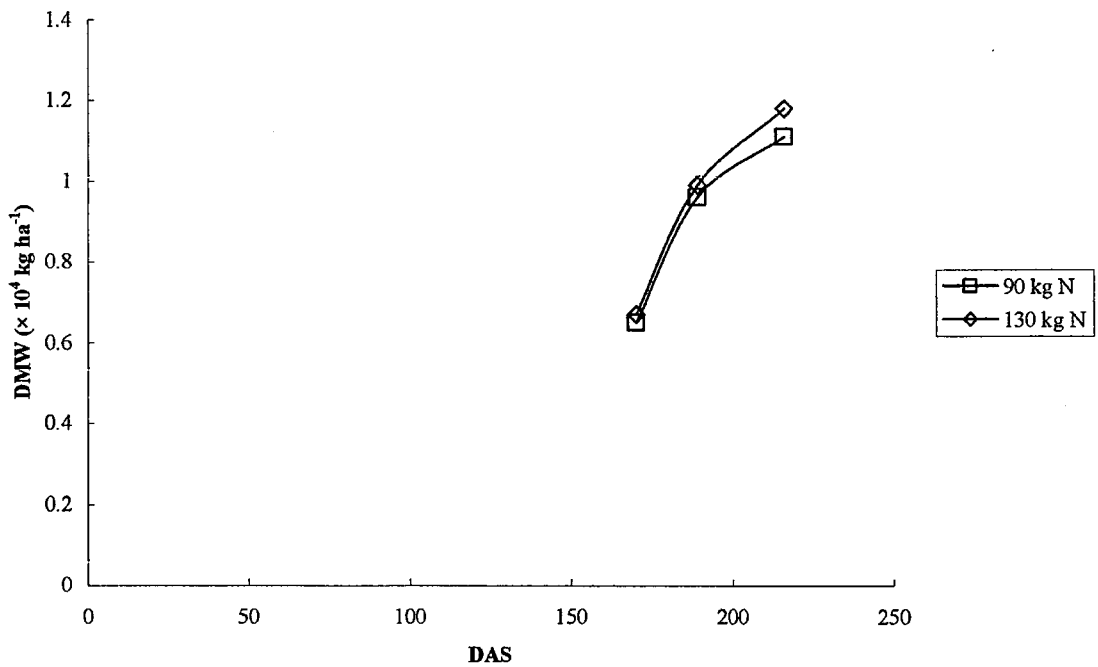


Figure 3.3.5 (d) The time change in aboveground DMW for N rates in Cycle II

kg N: kg ha^{-1} N

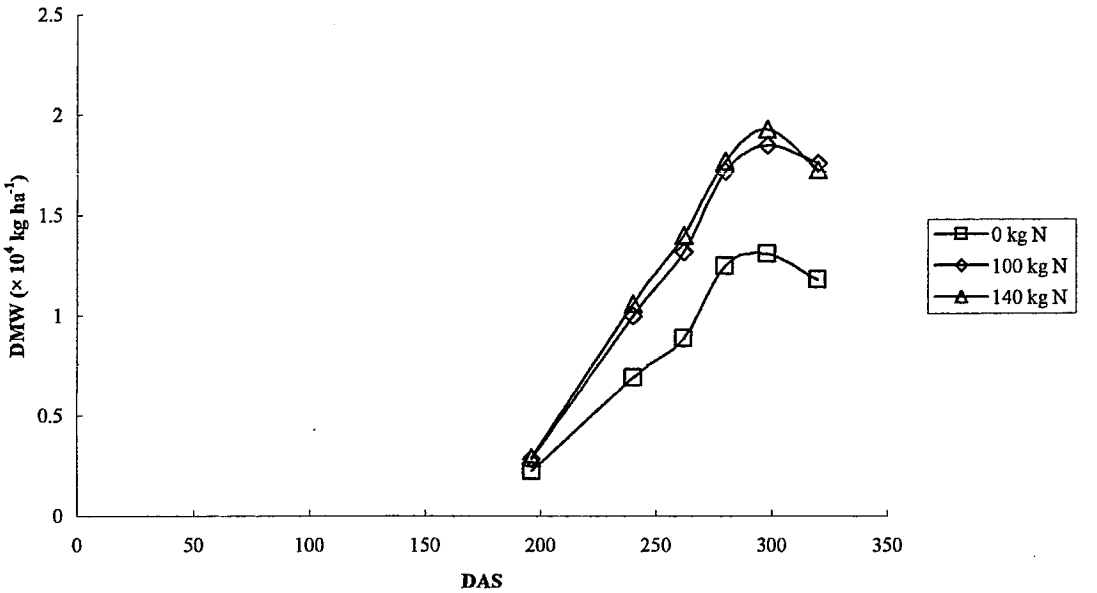


Figure 3.3.5 (e) The time change in aboveground DMW for N rates in Cycle III-B

kg N: kg ha^{-1} N

3.3.6 Intercepted PAR, Dry Matter Accumulation and RUE

Cycle III-B

A linear regression analysis was performed to study the relationship between intercepted PAR (estimated) and DM accumulation (measured) between GS 30/31 and pre-harvest (Fig. 3.3.6). 77 % of the variability in DM accumulation was accounted for by intercepted PAR (Table 3.3.6 (a)).

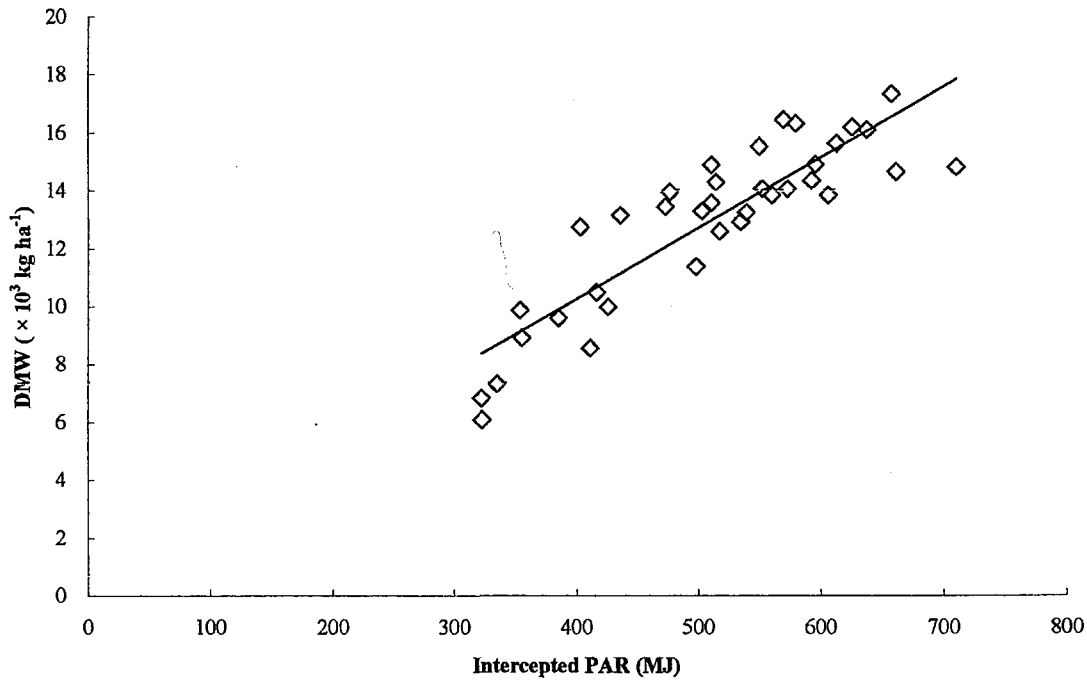


Figure 3.3.6 The relationship between simulated PAR interception and measured DMW in Cycle III-B

Table 3.3.6 (a) Coefficients of linear regression analysis of the relationship between simulated PAR interception and measured aboveground DMW at pre-harvest

Response Variable	*Coefficient (t pr.)*	*Constant (t pr.)*	P *value*	R^2
Aboveground DMW	2.43×10^{-2}	0.51	< 0.001	0.77
(× 10^3 kg ha^{-1})	(< 0.001)	(= 0.665)		

Radiation Use Efficiency (RUE) was estimated by using the following equation (Eq. 3.3.6). There was no interaction in RUE between fungicide programmes and N rates in Cycle III-B. RUE was not significantly affected by either fungicide programmes or N rates (Table 3.3.6 (b)).

RUE = (DM accumulation)/(Intercepted PAR) (Eq. 3.3.6)

RUE: Radiation Use Efficiency (g MJ^{-1})

Table 3.3.6 (b) RUE for fungicide programmes and N rates in Cycle III-B

Fungicide	*untrt*	*epoxi*	*epoxi + triflo*	P *value*	*CV %*
Programmes	2.64	2.53	2.43	= 0.220	11.1

N rates (kg ha^{-1})	**0**	**100**	**140**	***P* value**
	2.45	2.67	2.48	= 0.126

3.3.7 Plant Parts Dry Matter Weight and Dry Matter Partitioning

3.3.7.1 Dry Matter Weight of Plant Parts

Interactions

There was no interaction in DMW of plant parts in Cycle I and Cycle III-C at any sampling. In Cycle II, interactions were observed in DMW of spike, lower stem and lower leaf at 168 – 172 days after sowing (i.e. after anthesis), in DMW of chaff, lower stem and lower leaf at 186 – 193 days after sowing (i.e. at approximately three weeks after anthesis). For example, at 168 – 172 days after sowing, the two N rates did not make difference in DMW of spike for untreated plots, the plots treated with epoxiconazole alone and those treated with a mixture of epoxiconazole and trifloxystrobin, while for those treated with a mixture of epoxiconazole and kresoxim-methyl, the plots treated with the N rate of 130 kg ha^{-1} showed a greater DMW of spike than those treated with that of 90 kg ha^{-1} by 270 kg ha^{-1} (P = 0.023). For the plots treated with the N rate of 90 kg ha^{-1}, the DMW of spike appeared to have been reduced following the application of a mixture of epoxiconazole and kresoxim-methyl compared to untreated plots (Fig. 3.3.7.1 (a)). The interactions in DMW of lower stem and lower leaf were similar to that of spike and are given in Appendix 7. At 186 – 193

days after sowing, there was no difference in the DMW of rachis & chaff between the two N rates for untreated plots, the plots treated with epoxiconazole alone and those treated with a mixture of epoxiconazole and kresoxim-methyl, while for the plots treated with a mixture of epoxiconazole and trifloxystrobin, the plots treated with the N rate of 130 kg ha^{-1} showed a greater DMW of spike than those treated with that of 90 kg ha^{-1} by 220 kg ha^{-1} ($P = 0.042$) (Fig. 3.3.7.1 (b)). Similar interactions were observed in DMW of lower stem and lower leaf (Appendix 7). In Cycle III-B, interactions between fungicide programmes and N rates were observed at 295 – 302 days after sowing (i.e. at approximately six weeks after anthesis). For the plots that received no N fertilizer, untreated plots showed a greater DMW of spike than the plots treated with epoxiconazole alone and those treated with a mixture of epoxiconazole and trifloxystrobin by 1800 kg ha^{-1} and 900 kg ha^{-1} respectively and the plots treated with a mixture of epoxiconazole and trifloxystrobin showed a greater DMW of spike than those treated with epoxiconazole alone by 900 kg ha^{-1} ($P = 0.007$) (Fig. 3.3.7.1 (c)). However, for the plots treated with the N rate of 100 kg ha^{-1}, untreated plots showed a smaller DMW of spike than those treated with fungicides with the difference ranging from 900 kg ha-1 to 1200 kg ha^{-1} ($P = 0.007$) (Fig. 3.3.7.1 (c)). For the plots treated with the N rate of 140 kg ha^{-1}, the DMW of spike was greater for the plots treated with a mixture of epoxiconazole and trifloxystrobin than untreated plots and those treated with epoxiconazole alone by 1100 kg ha^{-1} and 600 kg ha^{-1} respectively ($P = 0.007$) (Fig. 3.3.7.1 (c)). For the plots where no N was applied, the application of fungicides no matter whether epoxiconazole alone or a mixture of epoxiconazole and trifloxystrobin appeared to have reduced the DMW of spike compared to untreated plots. The degree of reduction was greater for the plots treated with epoxiconazole alone than those treated with a mixture of epoxiconazole and trifloxystrobin by 900 kg ha^{-1} (Fig. 3.3.7.1 (c)).

As to stem, for the plots that received no N fertilizer, untreated plots showed a greater DMW of stem than those treated with epoxiconazole alone by 1110 kg ha^{-1} ($P = 0.004$) (Table 3.3.7.1 (d)). For the plots treated with the N rate of 100 kg ha^{-1}, the plots treated with epoxiconazole alone showed a greater DMW of stem than untreated plots by 860 kg ha^{-1} ($P = 0.004$) (Fig. 3.3.7.1 (d)). For the plots treated with the N rate of 140 kg ha^{-1}, the DMW of stem was greater for the plots treated with a mixture of epoxiconazole and trifloxystrobin than untreated plots by 970 kg ha^{-1} ($P = 0.004$) (Fig. 3.3.7.1 (d)). For the plots where no N was applied, the DMW of stem appeared to have been reduced following the application of epoxiconazole alone compared to untreated plots by 1110 kg ha^{-1} (Fig. 3.3.7.1 (d)).

The DMW of flag leaf was greater for untreated plots than the plots treated with epoxiconazole alone by 69 kg ha^{-1} with no application of N fertilizer, while there was no difference in DMW of flag leaf between fungicide treatments with the N application rate of 100 kg ha^{-1} ($P = 0.008$) (Fig. 3.3.7.1 (e)). With the N application rate of 140 kg ha^{-1}, the DMW of flag leaf was greater for the plots treated with a mixture of epoxiconazole and trifloxystrobin than untreated plots and those treated with epoxiconazole alone by 119 kg ha^{-1} and 108 kg ha^{-1} respectively ($P = 0.008$) (Fig. 3.3.7.1 (e)). For the plots where no N was applied, the DMW of flag leaf appeared to have been reduced following the application of epoxiconazole alone compared to untreated plots by 67 kg ha^{-1} (Fig. 3.3.7.1 (e)). Interactions in second leaf were similar to those in flag leaf ($P = 0.009$) (Fig. 3.3.7.1 (f)).

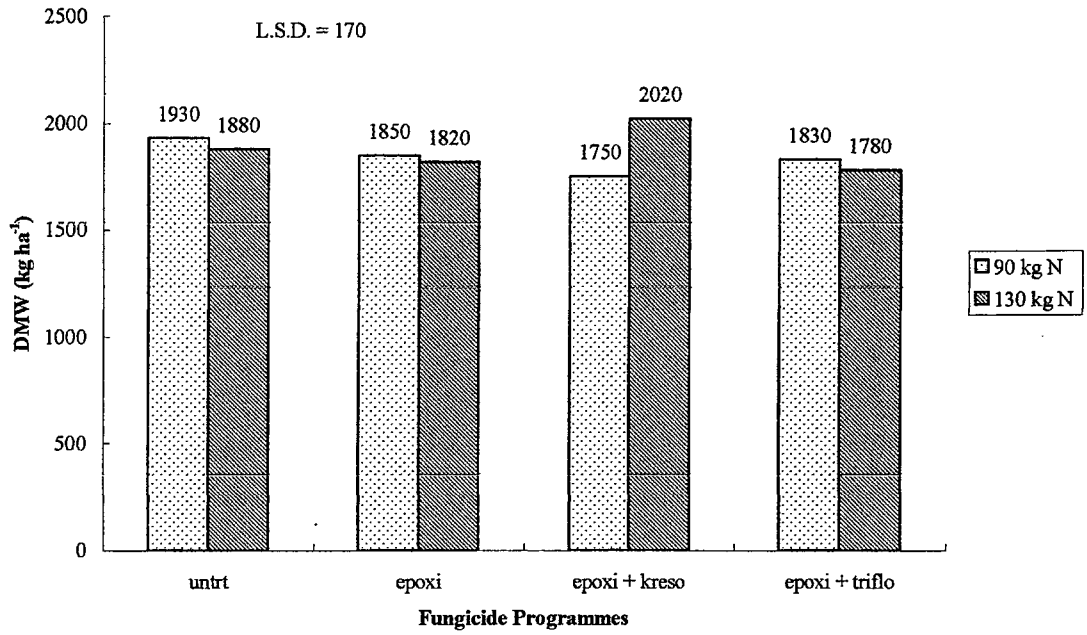


Figure 3.3.7.1 (a) The interaction in DMW of spike between fungicide programmes and N rates at 168 – 172 DAS (after anthesis) in Cycle II

untrt: untreated; epoxi: epoxiconazole; kreso: kresoxim-methyl; triflo: trifloxystrobin

kg N: kg N ha^{-1}

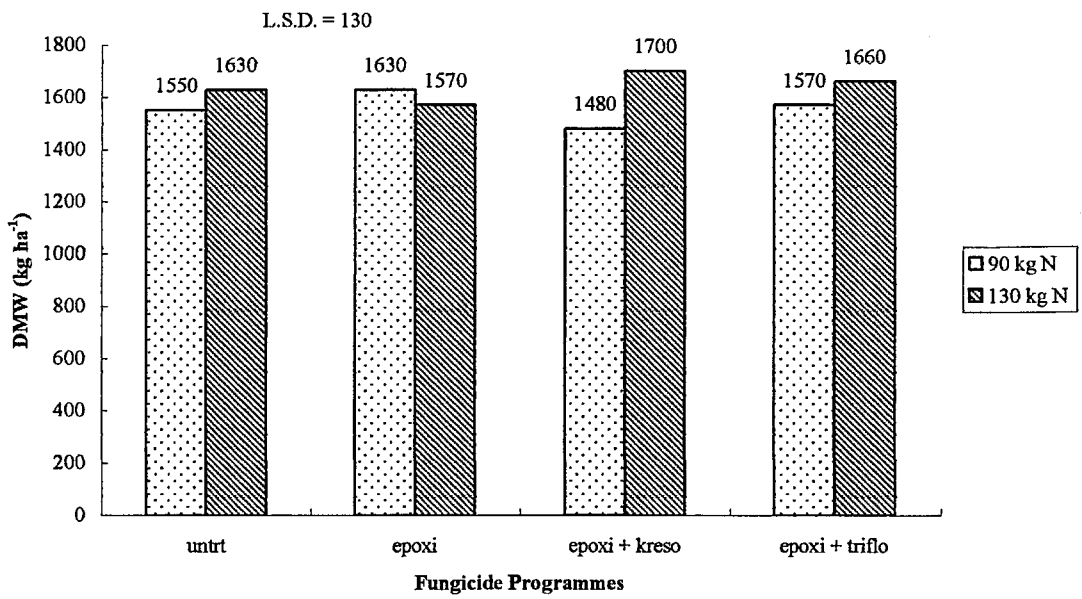


Figure 3.3.7.1 (b) The interaction in DMW of rachis & chaff between fungicide programmes and N rates at 186 – 193 DAS (after anthesis) in Cycle II

untrt: untreated; epoxi: epoxiconazole; kreso: kresoxim-methyl; triflo: trifloxystrobin

kg N: kg N ha^{-1}

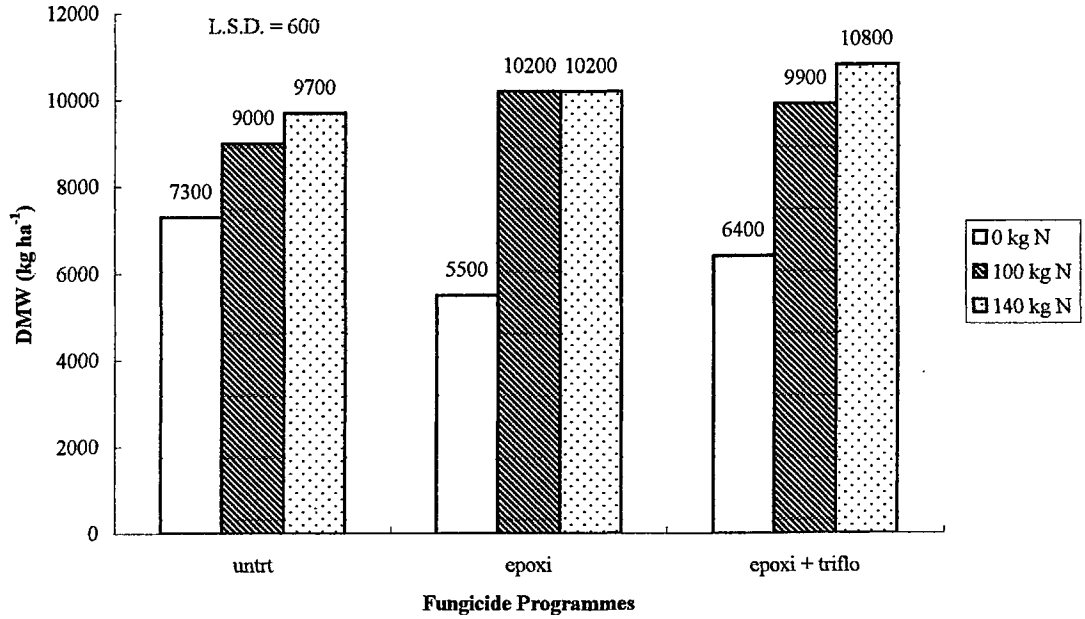


Figure 3.3.7.1 (c) The interaction in DMW of spike between fungicide programmes and N rates at 295 – 302 DAS (at approximately six weeks after anthesis) in Cycle III-B

untrt: untreated; epoxi: epoxiconazole; triflo: trifloxystrobin; kg N: kg N ha^{-1}

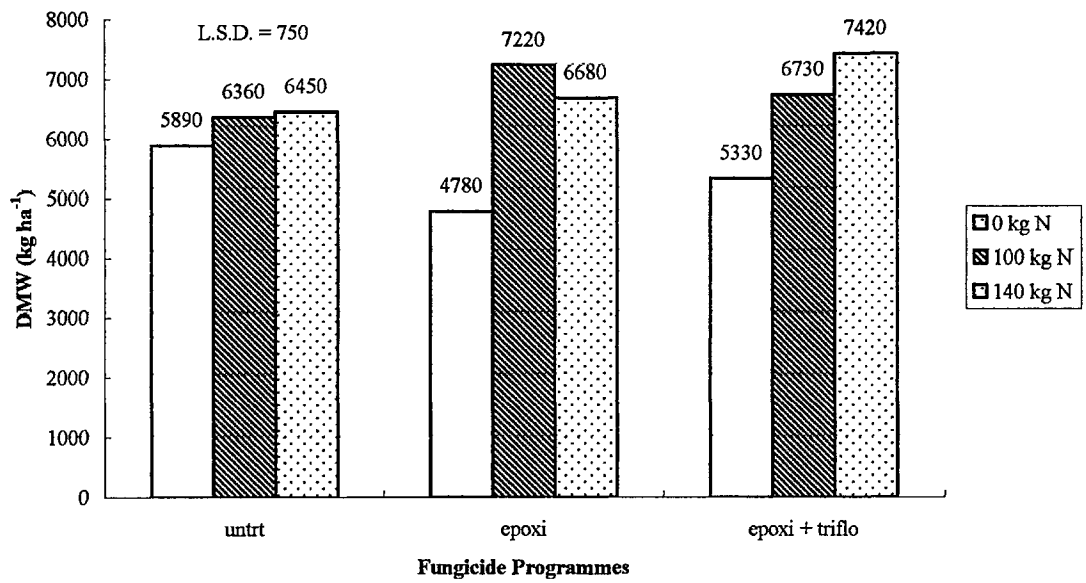


Figure 3.3.7.1 (d) The interaction in DMW of stem between fungicide programmes and N rates at 295 – 302 DAS (i.e. at approximately six weeks after anthesis) in Cycle III-B

untrt: untreated; epoxi: epoxiconazole; triflo: trifloxystrobin; kg N: kg N ha^{-1}

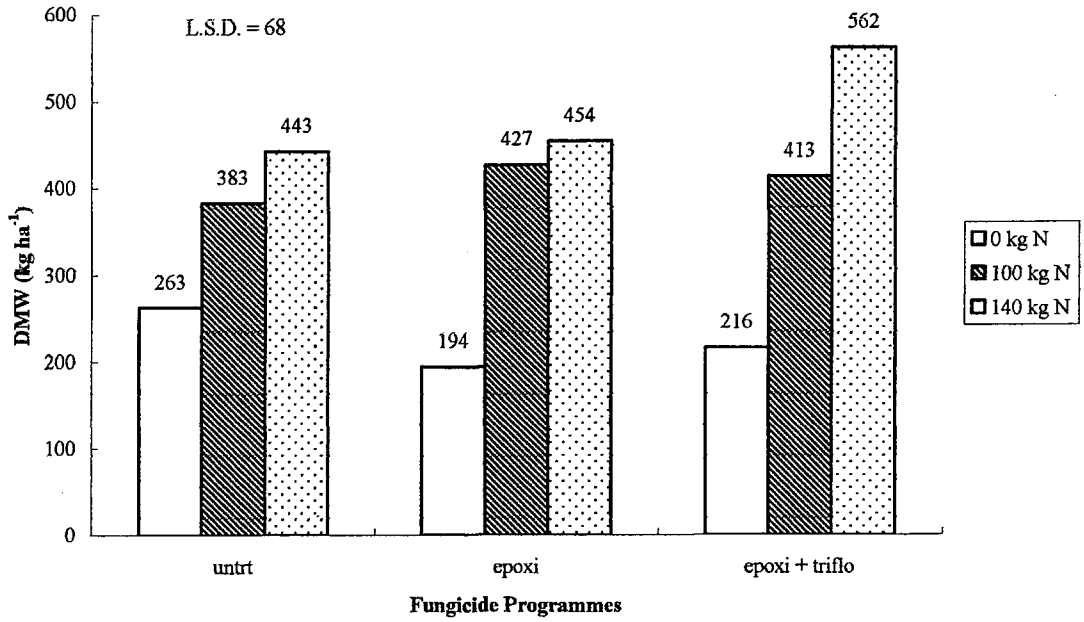


Figure 3.3.7.1 (e) The interaction in DMW of flag leaf between fungicide programmes and N rates at 295 – 302 DAS (at approximately six weeks after anthesis) in Cycle III-B

untrt: untreated; epoxi: epoxiconazole; triflo: trifloxystrobin; kg N: kg N ha^{-1}

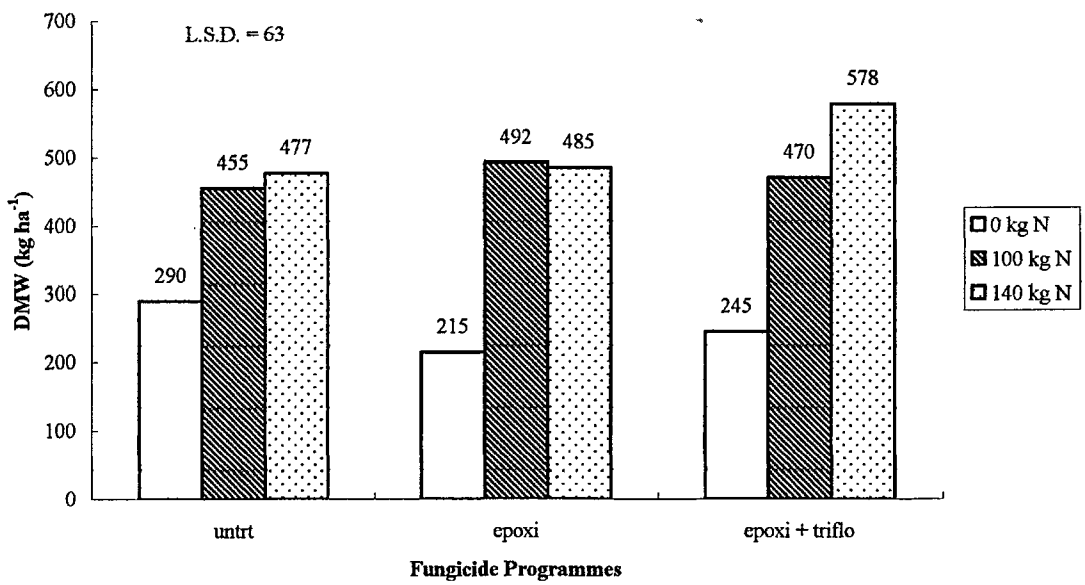


Figure 3.3.7.1 (f) The interaction in DMW of leaf 2 between fungicide programmes and N rates at 295 – 302 DAS (at approximately six weeks after anthesis) in Cycle III-B

untrt: untreated; epoxi: epoxiconazole; triflo: trifloxystrobin; kg N: kg N ha^{-1}

Fungicide Programmes

In Cycle I fungicide programmes did not affect DMW of stem, spike and senesced leaf, however, the DMW of green leaf was affected. At 229 – 232 days after sowing (i.e. at approximately two weeks before anthesis), the plots treated with a mixture of epoxiconazole and trifloxystrobin showed a greater DMW of total green leaf than untreated plots, those treated with epoxiconazole alone and those treated with a mixture of epoxiconazole and kresoxim-methyl by 220 kg ha^{-1}, 140 kg ha^{-1} and 230 kg ha^{-1} respectively ($P = 0.003$) (Table 3.3.7.1 (a)). At 241 – 246 days after sowing (i.e. at anthesis), the plots treated with a mixture of epoxiconazole and trifloxystrobin showed a greater DMW of total green leaf than untreated plots and those treated with epoxiconazole alone by 180 kg ha^{-1} and 120 kg ha^{-1} respectively ($P = 0.039$) (Table 3.3.7.1 (a)). At 257 – 259 days after sowing (i.e. at approximately two weeks after anthesis), untreated plots showed a smaller DMW of total green leaf than the plots treated with epoxiconazole alone and those treated with a mixture of epoxiconazole and trifloxystrobin by 140 kg ha^{-1} and 200 kg ha^{-1} respectively ($P = 0.041$) (Table 3.3.7.1 (a)). At 271 – 273 days after sowing (i.e. at approximately four weeks after anthesis), the plots treated with a mixture of epoxiconazole and trifloxystrobin showed a greater DMW of total green leaf than untreated plots, the plots treated with epoxiconazole alone and those treated with a mixture of epoxiconazole and kresoxim-methyl by 280 kg ha^{-1}, 190 kg ha^{-1} and 180 kg ha^{-1} respectively ($P < 0.001$) (Table 3.3.7.1 (a)).

In Cycle II fungicide programmes did not affect DMW of any plant parts. In Cycle III-B, fungicide programmes did not affect DMW of stem and spike but did affect DMW of green leaf and senesced leaf. At 255 – 264 days after sowing (i.e. at anthesis), untreated plots showed a smaller DMW of lower green leaf

than the plots treated with epoxiconazole alone and those treated with a mixture of epoxiconazole and trifloxystrobin by 90 kg ha^{-1} and 140 kg ha^{-1} respectively ($P = 0.004$) (Table 3.3.7.1 (b)). At 269 – 288 days after sowing (i.e. at approximately three weeks after anthesis), the plots treated with a mixture of epoxiconazole and trifloxystrobin showed a greater DMW of lower green leaf than untreated plots and the plots treated with epoxiconazole alone by 260 kg ha^{-1} and 180 kg ha^{-1} respectively and the plots treated with epoxiconazole alone showed a greater DMW of lower green leaf than untreated plots by 80 kg ha^{-1} ($P < 0.001$) (Table 3.3.7.1 (b)). The DMW of lower senesced leaf was smaller for the plots treated with a mixture of epoxiconazole and trifloxystrobin than for untreated plots and those treated with epoxiconazole alone by 210 kg ha^{-1} and 110 kg ha^{-1} respectively ($P = 0.002$) (Table 3.3.7.1 (b)). At 295 – 302 days after sowing (i.e. at approximately six weeks after anthesis), the plots treated with a mixture of epoxiconazole and trifloxystrobin showed a greater DMW of lower green leaf than untreated plots and those treated with epoxiconazole alone by 52 kg ha^{-1} and 33 kg ha^{-1} respectively and the plots treated with epoxiconazole showed a greater DMW of lower green leaf than untreated plots by 19 kg ha^{-1} ($P < 0.001$) (Table 3.3.7.1 (b)).

In Cycle III-C at pre-harvest, the plots treated with a mixture of epoxiconazole and trifloxystrobin showed a greater DMW of vegetative plant than untreated plots and the plots treated with epoxiconazole alone by 710 kg ha^{-1} and 400 kg ha^{-1} respectively and the plots treated with a mixture of epoxiconazole and kresoxim-methyl showed a greater DMW of vegetative plant than untreated plots by 460 kg ha^{-1} ($P = 0.008$) (Table 3.3.7.1 (c)). The DMW of rachis and chaff was smaller for untreated plots than the plots treated with fungicides with the difference ranging from 150 kg ha^{-1} to 200 kg ha^{-1} ($P = 0.002$) (Table 3.3.7.1 (c)).

Table 3.3.7.1 (a) DMW of total green leaf (kg ha^{-1}) for fungicide programmes in Cycle I

DAS	*untrt*	*epoxi*	*epoxi + kreso*	*epoxi + triflo*	*P value*	*L.S.D.*	*CV %*
229 – 232	1770	1850	1760	1990	= 0.003	120	6.6
241 – 246	1750	1810	1830	1930	= 0.039	120	6.6
257 – 259	1320	1460	1390	1520	= 0.041	140	9.7
271 – 273	740	830	840	1020	< 0.001	110	13.0

untrt: untreated; epoxi: epoxiconazole; kreso: kresoxim-methyl; triflo: trifloxystrobin

Table 3.3.7.1 (b) DMW of total green leaf and senesced leaf (kg ha^{-1}) for fungicide programmes in Cycle III-B

DAS	*Leaf*	*untrt*	*epoxi*	*epoxi + triflo*	*P value*	*L.S.D.*	*CV %*
255 – 264	*LG Leaf*	510	600	650	= 0.004	70	15.0
269 – 288	*LG Leaf*	160	240	420	< 0.001	80	34.0
	L Sen Leaf	1180	1080	970	= 0.002	110	11.8
295 – 302	*LG Leaf*	2	21	54	< 0.001	16	72.5

untrt: untreated; epoxi: epoxiconazole; kreso: kresoxim-methyl; triflo: trifloxystrobin

LG Leaf: lower green leaf; L Sen Leaf: lower senesced leaf

Table 3.3.7.1 (c) DMW of vegetative plant and rachis and chaff (kg ha^{-1}) for fungicide programmes at pre-harvest in Cycle III-C

DAS	*Plant Parts*	*untrt*	*epoxi*	*epoxi + kreso*	*epoxi + triflo*	*P value*	*L.S.D.*	*CV %*
314 - 317	*Vegetative*	7980	8290	8440	8690	= 0.008	400	6.7
	Rachis + Chaff	1890	2070	2040	2090	= 0.002	110	7.8

untrt: untreated; epoxi: epoxiconazole; kreso: kresoxim-methyl; triflo: trifloxystrobin

3.3.7.2 Dry Matter Partitioning

The percentage of DM partitioning to each plant part was calculated using the following equation (Eq. 3.3.7.2)

$$DMP = (DMWp/DMWt) \times 100 \qquad \text{(Eq. 3.3.7.2)}$$

DMP: the percentage of DM partitioning of a given plant part

DMWp: the DMW of a given plant part

DMWt: the DMW of total (aboveground) weight

Interactions

In Cycle I and Cycle III-B, there was no interaction in DMP of any plant part between fungicide programmes and N rates at any sampling time. In Cycle II interactions were observed in DMP of upper stem and lower stem between fungicide programmes and N rates at 186 – 193 days after sowing (i.e. at approximately three weeks after anthesis) and 214 – 219 days after sowing (i.e. at approximately six weeks after anthesis) respectively (Fig. 3.3.7.2 (a)). At 186 – 193 days after sowing, the two N rates gave no difference in DMP of upper stem for untreated plots and the plots treated with epoxiconazole alone, while they did for the plots treated with strobilurin fungicide programmes ($P = 0.043$) (Fig. 3.3.7.2 (a)). As to the plots treated with a mixture of epoxiconazole and kresoxim-methyl, the plots treated with the N rate of 90 kg ha^{-1} showed a greater DMP of upper stem than those treated with that of 130 kg ha^{-1} by 0.7 % ($P = 0.043$) (Fig. 3.3.7.2 (a)).

Contrary to this, for the plots treated with a mixture of epoxiconazole and trifloxystrobin, the plots treated with the N rate of 90 kg ha^{-1} showed a smaller DMP of upper stem than those treated with that of 130 kg ha^{-1} by 0.7 % ($P = 0.043$) (Fig. 3.3.7.2 (a)).

At 214 – 219 days after sowing, the two N rates did not make difference in DMP of lower stem for untreated plots, the plots treated with epoxiconazole alone and those treated with a mixture of epoxiconazole and kresoxim-methyl, while they did for the plots treated with a mixture of epoxiconazole and trifloxystrobin where the plots treated with the N rate of 90 kg ha^{-1} showed a greater DMP of lower stem than those treated with that of 130 kg ha^{-1} by 1.09 % ($P = 0.003$) (Fig. 3.3.7.2 (b)).

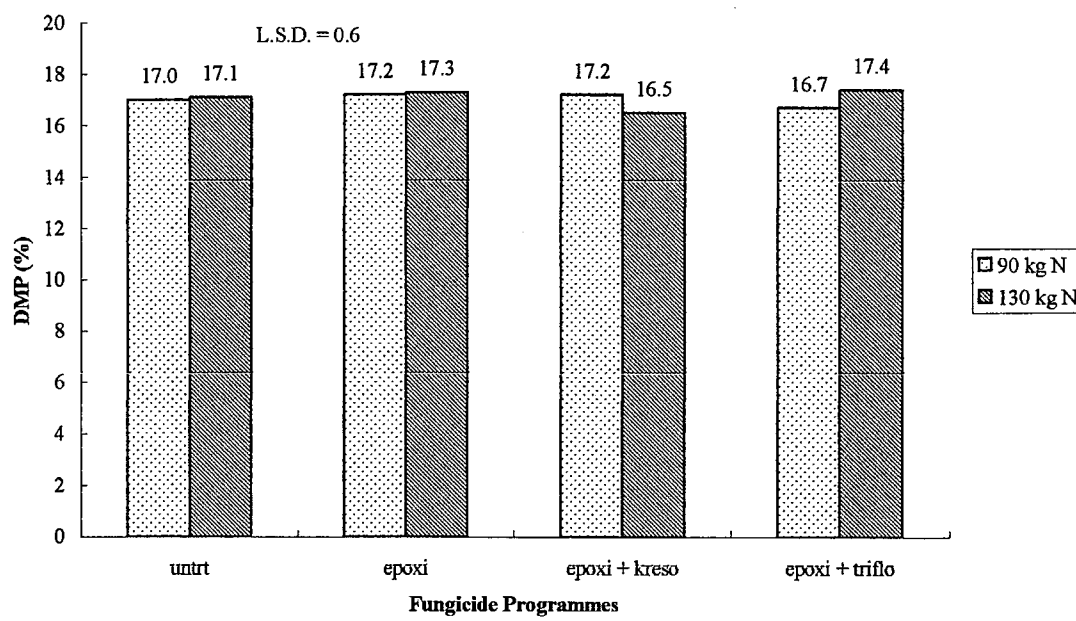


Figure 3.3.7.2 (a) The interaction in DMP of upper stem between fungicide programmes and N rates at 186 – 193 DAS (approximately three weeks after anthesis) in Cycle II

untrt: untreated; epoxi: epoxiconazole; kreso: kresoxim-methyl; triflo: trifloxystrobin

kg N: kg N ha^{-1}

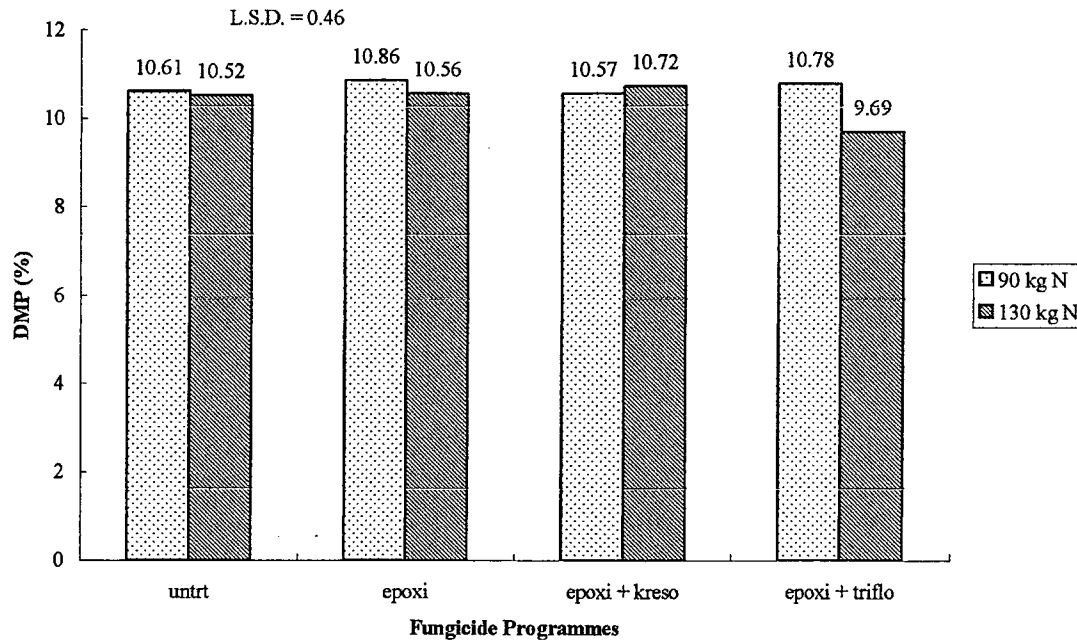


Figure 3.3.7.2 (b) The interaction in DMP of lower stem between fungicide programmes and N rates at 214 – 219 DAS (approximately six weeks after anthesis) in Cycle II

untrt: untreated; epoxi: epoxiconazole; kreso: kresoxim-methyl; triflo: trifloxystrobin

kg N: kg N ha^{-1}

Fungicide Programmes

In Cycle I at 214 – 216 days after sowing (i.e. at approximately four weeks before anthesis), the DMP of stem was smaller for the plots treated with a mixture of epoxiconazole and trifloxystrobin than for untreated plots, the plots treated with epoxiconazole alone and those treated with a mixture of epoxiconazole and kresoxim-methyl by 0.8 %, 1.1 % and 0.9 % respectively ($P = 0.025$) (Table 3.3.7.2 (a)).

The DMP of green leaf was greater for the plots treated with a mixture of epoxiconazole and trifloxystrobin than for untreated plots and the plots treated with epoxiconazole alone by 1.7 % and 1.6 % respectively ($P = 0.012$) (Table 3.3.7.2 (a)). At 229 – 232 days after sowing (i.e. at approximately two weeks before anthesis), the plots treated with a mixture of epoxiconazole and trifloxystrobin showed a greater DMP of green leaf

than untreated plots and those treated with epoxiconazole alone by 1.2 % and 0.8 % respectively ($P = 0.026$) (Table 3.3.7.2 (a)). At 241 – 246 days after sowing (i.e. at anthesis), the plots treated with a mixture of epoxiconazole and kresoxim-methyl showed a greater DMP of green leaf than untreated plots and the plots treated with epoxiconazole alone by 0.7 % and 0.5 % respectively ($P = 0.002$) (Table 3.3.7.2 (a)). Similarly, the plots treated with a mixture of epoxiconazole and trifloxystrobin showed a greater DMP of green leaf than untreated plots and the plots treated with epoxiconazole alone by 1.0 % and 0.8 % respectively ($P = 0.002$) (Table 3.3.7.2 (a)). At 271 – 273 days after sowing (i.e. at approximately four weeks after anthesis), untreated plots showed a smaller DMP of green leaf than the plots treated with fungicides with the difference ranging from 0.44 % to 1.15 % ($P < 0.001$) (Table 3.3.7.2 (a)). The plots treated with a mixture of epoxiconazole and kresoxim-methyl showed a greater DMP of green leaf than the plots treated with epoxiconazole alone and those treated with a mixture of epoxiconazole and kresoxim-methyl by 0.69 % and 0.49 % respectively ($P < 0.001$) (Table 3.3.7.2 (a)). At the same time, the DMP of senesced leaf was greater for untreated plots than the plots treated with a mixture of epoxiconazole and trifloxystrobin by 1.0 % ($P = 0.032$) (Table 3.3.7.2 (a)).

In Cycle II at 168 – 172 days after sowing (i.e. after anthesis), untreated plots showed a greater DMP of upper stem than the plots treated with a mixture of epoxiconazole and kresoxim-methyl and those treated with a mixture of epoxiconazole and trifloxystrobin by 0.5 % and 0.8 % respectively ($P = 0.037$) (Table 3.3.7.2 (b)). The DMP of lower stem was greater for the plots treated with a mixture of epoxiconazole and kresoxim-methyl than for untreated plots and those treated with a mixture of epoxiconazole and

trifloxystrobin by 0.7 % and 0.8 % respectively (P = 0.018) (Table 3.3.7.2 (b)). Untreated plots showed a smaller DMP of lower leaf than the plots treated with a mixture of epoxiconazole and kresoxim-methyl and those treated with a mixture of epoxiconazole and trifloxystrobin by 0.5 % and 0.9 % respectively and the plots treated with a mixture of epoxiconazole and trifloxystrobin showed a greater DMP of lower leaf than those treated with epoxiconazole alone by 0.5 % (P = 0.003) (Table 3.3.7.2 (b)). At 186 – 193 days after sowing (i.e. at approximately three weeks after anthesis), untreated plots and the plots treated with a mixture of epoxiconazole and trifloxystrobin showed a smaller DMP of lower stem than those treated with epoxiconazole alone and those treated with a mixture of epoxiconazole and kresoxim-methyl by 0.5 % and 0.6 % respectively (P = 0.026) (Table 3.3.7.2 (b)). The DMP of lower leaf was smaller for untreated plots than for those treated with fungicides with the difference ranging from 0.23 % to 0.25 % (P = 0.032) (Table 3.3.7.2 (b)). At 214 – 219 days after sowing (i.e. at approximately six weeks after anthesis), untreated plots showed a greater DMP of upper stem than the plots treated with epoxiconazole alone and those treated with a mixture of epoxiconazole and trifloxystrobin by 0.26 % and 0.41 % respectively (P = 0.023) (Table 3.3.7.2 (b)). The plots treated with a mixture of epoxiconazole and trifloxystrobin showed a smaller DMP of lower stem than untreated plots, those treated with epoxiconazole alone and those treated with a mixture of epoxiconazole and kresoxim-methyl by 0.34 %, 0.48 % and 0.41 % respectively (P = 0.026) (Table 3.3.7.2 (b)).

In Cycle III-B, at 194 – 198 days after sowing, untreated plots showed a smaller DMP of green leaf than the plots treated with a mixture of epoxiconazole and trifloxystrobin by 1.3 % (P = 0.045) (Table 3.3.7.2 (c)).

At 255 – 264 days after sowing (i.e. at anthesis), untreated plots showed a greater DMP of stem than the plots treated with epoxiconazole alone and those treated with a mixture of epoxiconazole and trifloxystrobin by 0.6 % and 0.9 % respectively ($P = 0.013$) (Table 3.3.7.2 (c)). Untreated plots showed a smaller DMP of lower green leaf than the plots treated with epoxiconazole alone and those treated with a mixture of epoxiconazole and trifloxystrobin by 0.76 % and 1.07 % respectively ($P < 0.001$) (Table 3.3.7.2 (c)). At 269 – 288 days after sowing (i.e. at approximately three weeks after anthesis), untreated plots showed a smaller DMP of lower green leaf than the plots treated with epoxiconazole alone and those treated with a mixture of epoxiconazole and trifloxystrobin by 0.7 % and 1.5 % respectively and the plots treated with epoxiconazole alone showed a smaller DMP of lower green leaf than those treated with a mixture of epoxiconazole and trifloxystrobin by 0.6 % ($P < 0.001$) (Table 3.3.7.2 (c)). The DMP of senesced leaf was smaller for the plots treated with a mixture of epoxiconazole and trifloxystrobin than untreated plots and those treated with epoxiconazole alone by 1.8 % and 1.5 % respectively ($P = 0.002$) (Table 3.3.7.2 (c)). At 295 – 302 days after sowing (i.e. at approximately six weeks after anthesis), untreated plots showed a smaller DMP of lower green leaf than the plots treated with epoxiconazole alone and those treated with a mixture of epoxiconazole and trifloxystrobin by 0.13 % and 0.30 % respectively and the plots treated with epoxiconazole alone showed a smaller DMP of lower green leaf than those treated with a mixture of epoxiconazole and trifloxystrobin by 0.17 % ($P < 0.001$) (Table 3.3.7.2 (c)).

Table 3.3.7.2 (a) The percentage of DMW of plant parts in the aboveground DMW for fungicide programmes in Cycle I

DAS	*Plant Part*	*untrt*	*epoxi*	*epoxi + kreso*	*epoxi + triflo*	*P value*	*L.S.D.*	*CV %*
214 – 216	*Stem*	62.9	63.2	63.0	62.1	= 0.025	0.7	1.2
	G Leaf	29.3	29.4	30.0	31.0	= 0.012	1.1	3.6
229 – 232	*G Leaf*	17.6	18.0	18.1	18.8	= 0.026	0.8	4.5
241 – 246	*G Leaf*	8.4	8.6	9.1	9.4	= 0.002	0.5	6.0
271 – 273	*G Leaf*	0.60	1.06	1.26	1.75	< 0.001	0.37	32.2
	Sen Leaf	9.5	9.0	8.9	8.5	= 0.032	0.7	7.4

untrt: untreated; epoxi: epoxiconazole; kreso: kresoxim-methyl; triflo: trifloxystrobin

G Leaf: Green Leaf; Sen Leaf: Senesced Leaf

Table 3.3.7.2 (b) The percentage of DMW of plant parts to the aboveground DMW for fungicide programmes in Cycle II

DAS	*Plant Part*	*untrt*	*epoxi*	*epoxi + kreso*	*epoxi + triflo*	*P value*	*L.S.D.*	*CV %*
168 – 172	*U Stem*	24.0	23.6	23.5	23.2	= 0.037	0.5	3.3
	L Stem	30.1	30.5	30.8	30.0	= 0.018	0.6	2.9
	L Leaf	7.9	8.3	8.4	8.8	= 0.003	0.5	8.6
186 – 193	*L Stem*	16.9	17.4	17.4	16.8	= 0.026	0.5	4.1
	L Leaf	4.94	5.18	5.17	5.19	= 0.032	0.19	5.5
214 – 219	*U Stem*	10.93	10.67	10.75	10.52	= 0.023	0.26	3.6
	L Stem	10.57	10.71	10.64	10.23	= 0.026	0.33	4.6

untrt: untreated; epoxi: epoxiconazole; kreso: kresoxim-methyl; triflo: trifloxystrobin

U Stem: Upper Stem; L Stem: Lower Stem; U Leaf: Upper Leaf; L Leaf: Lower Leaf

Table 3.3.7.2 (c) The percentage of DMW of plant parts to the aboveground DMW for fungicide programmes in Cycle III-B

DAS	*Plant Part*	*untrt*	*epoxi*	*epoxi + triflo*	*P value*	*L.S.D.*	*CV %*
194 – 198	*G Leaf*	39.6	40.0	40.9	= 0.045	1.1	3.1
255 – 264	*Stem*	65.8	65.2	64.9	= 0.013	0.6	1.1
	L G Leaf	4.17	4.93	5.24	< 0.001	0.44	10.9
269 – 288	*L G Leaf*	1.1	1.8	2.6	< 0.001	0.6	39.0
	Sen Leaf	7.7	7.4	5.9	= 0.002	1.0	16.7
295 – 302	*L G Leaf*	0.01	0.14	0.31	< 0.001	0.10	76.3

untrt: untreated; epoxi: epoxiconazole;. triflo: trifloxystrobin

G Leaf: green leaf; L G Leaf: Lower green leaf; Sen Leaf: Senesced leaf

3.3.8 Yield and Yield Components

3.3.8.1 Combine-harvested Grain Yield

In the following section, combine-harvested grain yield is presented for each field experiment except for Cycle II where manually-harvested grain yield is shown due to a number of gaps found in some of the plots.

Interactions

In Cycle I and Cycle II, there was no interaction in yield between fungicide programmes and N rates. In Cycle III-B, there was an interaction in yield of combine-harvested grains at harvest. There was no difference in yield between fungicide programmes in absence of N fertilization, while yield was lower for untreated plots with the N application rate of 100 kg ha^{-1} than for the plots treated with a mixture of epoxiconazole and trifloxystrobin by 1300 kg ha^{-1} ($P = 0.045$) (Fig. 3.3.8.1 (a)). With the N application rate

of 140 kg ha^{-1}, untreated plots showed a lower yield than those treated with epoxiconazole alone and those treated with a mixture of epoxiconazole and trifloxystrobin by 1200 kg ha^{-1} and 1500 kg ha^{-1} respectively (P = 0.045) (Fig. 3.3.8.1 (a)). In Cycle III-C, interactions in yield of combine-harvested grains were observed between varieties and fungicide programmes. Equinox showed a greater yield than Hereward with the application of fungicides, while the opposite was observed for untreated plots with the difference being 780 kg ha^{-1} ($P < 0.001$) (Fig. 3.3.8.1 (b)). In the presence of fungicides, the difference in yield between the two varieties ranged from 910 kg ha^{-1} to 1590 kg ha^{-1} (Fig. 3.3.8.1 (b)).

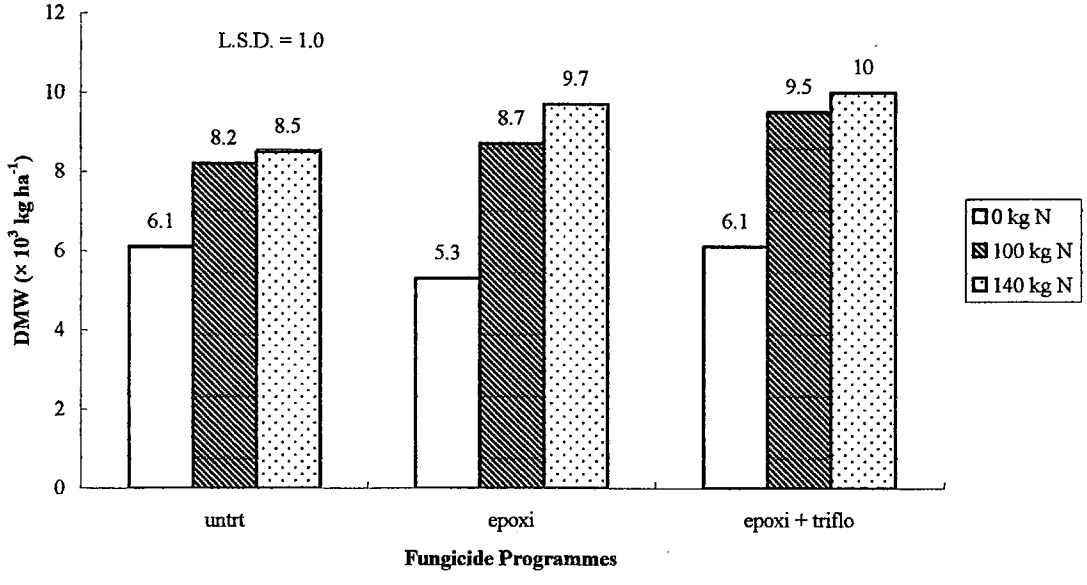


Figure 3.3.8.1 (a) The interaction in yield of combine-harvested grains (@ 85 % DM) at harvest between fungicide programmes and N rates in Cycle III-B

untrt: untreated; epoxi: epoxiconazole; triflo: trifloxystrobin

kg N: kg N ha^{-1}

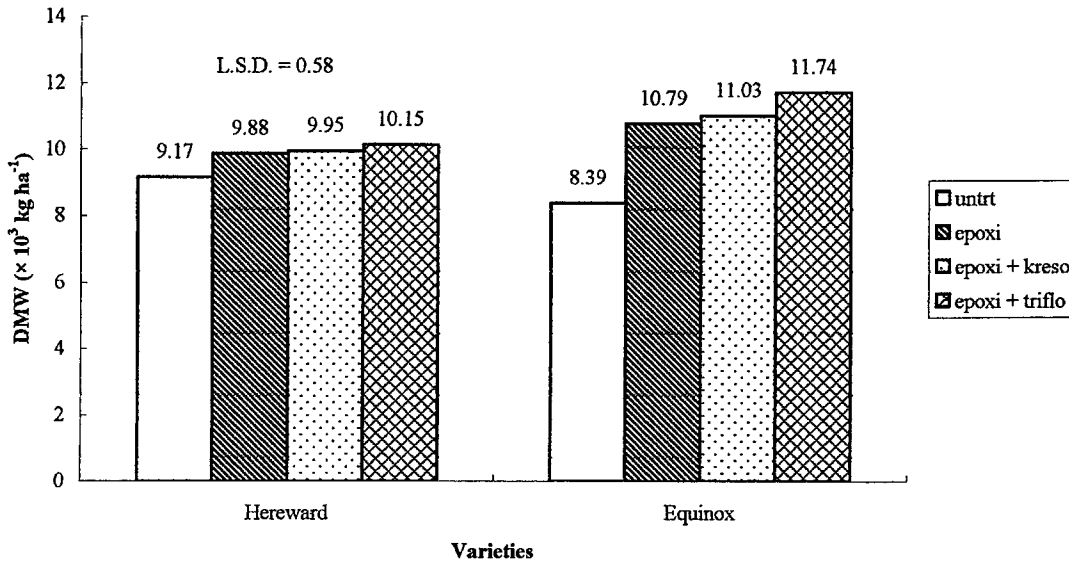


Figure 3.3.8.1 (b) The interaction in yield of combine-harvested grains (@ 85 % DM) at harvest between varieties and fungicide programmes in Cycle III-C

untrt: untreated; epoxi: epoxiconazole; kreso: kresoxim-methyl; triflo: trifloxystrobin

Fungicide Programmes

In Cycle I no difference was observed in grain yield between fungicide programmes (Table 3.3.8.1 (a)). There was no difference in yield for manually-harvested grain yield in Cycle II (Table 3.3.8.1 (a)). In Cycle III-B, the plots treated with a mixture of epoxiconazole and trifloxystrobin showed a greater yield compared to untreated plots and those treated with epoxiconazole alone by 900 kg ha^{-1} and 600 kg ha^{-1} respectively (P = 0.006) (Table 3.3.8.1 (a)). In Cycle III-C untreated plots showed a lower yield than those treated with fungicides ($P < 0.001$) (Table 3.3.8.1 (a)). The difference ranged from 1560 kg ha^{-1} to 2170 kg ha^{-1} ($P <$ 0.001) (Table 3.3.8.1 (a)). In addition, the plots treated with a mixture of epoxiconazole and trifloxystrobin showed a greater grain yield compared to those treated with epoxiconazole alone by 610 kg ha^{-1} ($P < 0.001$) (Table 3.3.8.1 (a)).

Table 3.3.8.1 (a) Combine-harvested grain yield (@ 85 % DM) for fungicide programmes ($\times 10^3$ kg ha^{-1})

Fungicide Programmes	*Cycle I*	*Cycle II**	*Cycle III-B*	*Cycle III-C*
untrt	8.20	7.5	7.6	8.78
epoxi	8.20	7.6	7.9	10.34
epoxi + kreso	8.29	7.5	-	10.49
epoxi + triflo	8.54	7.5	8.5	10.95
P value	= 0.077	= 0.936	= 0.006	< 0.001
L.S.D.	NS	NS	0.6	0.41
CV %	3.6	10.3	8.2	5.7

untrt: untreated; epoxi: epoxiconazole; kreso: kresoxim-methyl; triflo: trifloxystrobin

*manually-harvested yield

N rates

In Cycle I and Cycle III-B the plots that received no N fertilizer showed lower yields compared to those that received N fertilizer ($P < 0.001$) (Table 3.3.8.1 (b)). The difference ranged from 4100 kg ha^{-1} to 4200 kg ha^{-1} in Cycle I and from 3000 kg ha^{-1} to 3800 kg ha^{-1} in Cycle III-B ($P < 0.001$) (Table 3.3.8.1 (b)). In both of the field experiments, the plots treated with the N rate of 140 kg ha^{-1} showed a greater yield than those treated with that of 100 kg ha^{-1} by 980 kg ha^{-1} in Cycle I ($P < 0.001$) and by 600 kg ha^{-1} in Cycle III-B ($P < 0.001$). In Cycle II for manually-harvested grains, the plots treated with the N rate of 130 kg ha^{-1} showed a greater yield than those treated with that of 90 kg ha^{-1} by 500 kg ha^{-1} ($P = 0.003$) (Table 3.3.8.1 (b)). In Cycle III-C the plots treated with the N rate of 200 kg ha^{-1} showed a greater yield than those treated with that of 100 kg ha^{-1} by 420 kg ha^{-1} ($P = 0.005$) (Table 3.3.8.1 (b)).

Table 3.3.8.1 (b) Combine-harvested grain yield (@ 85 % DM) for N rates (× 10^3 kg ha^{-1})

N rates (kg ha^{-1})	*Cycle I*	*Cycle II**	*Cycle III-B*	*Cycle III-C*
0	5.38		5.8	-
90		7.3		
100	9.28	-	8.8	9.93
130		7.8		
140	10.26	-	9.4	-
200	-	-	-	10.35
P value	< 0.001	= 0.003	< 0.001	= 0.005
L.S.D.	0.25	0.4	0.6	0.29
CV %	3.6	10.3	8.2	5.7

*manually-harvested yield

3.3.8.2 Tiller Number per Area (TNA)

Cycle III-B

The plots that received no N fertilizer showed the lowest TNA compared to those treated with N throughout the period from GS 31/32 until pre-harvest (Table 3.3.8.2). No difference in TNA was observed between the plots treated with the N rate of 100 kg ha^{-1} and that of 140 kg ha^{-1} (Table 3.3.8.2). At the sampling conducted at 194 – 198 days after sowing (i.e. at approximately nine weeks before anthesis), it was recognized that the range of 6.0 to 16.9 % of tillers were affected by gout flies (Photograph 8 and 9). These tillers were excluded from effective tillers. Covariate analysis with gout fly incidence was significant (P = 0.014) (Table 3.3.8.2). From the next sampling conducted at 237 – 244 days after sowing (i.e. at approximately two weeks before anthesis), the tillers affected by gout flies were decayed and thus were not recognized very clearly. Between the period from 194 – 198 days after sowing (i.e. at approximately nine weeks before anthesis) until 237 – 244 days after sowing (i.e. at approximately two weeks before anthesis),

the number of effective tillers declined by approximately 50 % for the plots that received no N fertilizer, while the reduction rate was slightly smaller for the plots treated with fertilizer N (Table 3.3.8.2). After GS 49 (237 – 244 DAS), TNA hardly changed until pre-harvest (311 – 323 DAS) irrespective of N rates (Table 3.3.8.2).

Table 3.3.8.2 Change in effective TNA (m^{-2}) from GS 31 to pre-harvest in Cycle III-B

N rates ($kg\ ha^{-1}$)	*194-198*	*237-244* *(GS 49)*	*255-264* *(GS 65)*	*269-288*	*295-302*	*311-323*
0	670	334	343	353	362	350
100	730	477	461	484	460	456
140	750	511	490	483	467	459
P value	= 0.016	< 0.001	< 0.001	< 0.001	< 0.001	= 0.013
P value *(covariate)**	= 0.014	-	-	-	-	-
L.S.D.	50	36	28	25	27	14
CV %	8.8	9.8	7.7	6.8	7.5	5.9

* the number of tillers affected by gout flies

3.3.8.3 Ear Number per Area (ENA)

Interactions

No interaction in ENA between treatments/factors was observed in any of the field experiments.

Fungicide Programmes

No difference in ENA between fungicide programmes was observed except in Cycle III-C (Table 3.3.8.3 (a)). In Cycle III-C, untreated plots showed a smaller ENA than the plots treated with epoxiconazole alone and those treated with a mixture of epoxiconazole and trifloxystrobin by 21 m^{-2} and 32 m^{-2} respectively (P =

0.016) (Table 3.3.8.3 (a)). ENA differed between strobilurin fungicide programmes. The plots treated with a mixture of epoxiconazole and kresoxim-methyl showed a smaller ENA than those treated with a mixture of epoxiconazole and trifloxystrobin by 21 m^{-2} ($P = 0.016$) (Table 3.3.8.3 (a)).

Table 3.3.8.3 (a) ENA (m^{-2}) for fungicide programmes at pre-harvest

Fungicide Programmes	*Cycle I*	*Cycle II*	*Cycle III-B*	*Cycle III-C*
untrt	445	362	423	455
epoxi	447	362	414	476
epoxi + kreso	430	353	-	466
epoxi + triflo	450	376	428	487
P value	= 0.201	= 0.235	= 0.586	= 0.016
L.S.D.	NS	NS	NS	20
CV %	4.6	9.3	8.3	5.9

untrt: untreated; epoxi: epoxiconazole; kreso: kresoxim-methyl; triflo: trifloxystrobin

N rates

Both in Cycle I and Cycle III-B the plots that received no N fertilizer showed a smaller ENA than those that received N fertilizer with the difference ranging from 46 m^{-2} to 48 m^{-2} in Cycle I and from 106 m^{-2} to 109 m^{-2} in Cycle III-B respectively ($P < 0.001$), while no difference was observed between the N rate of 100 kg ha^{-1} and that of 140 kg ha^{-1} (Table 3.3.8.3 (b)). In Cycle II the plots treated with the N rate of 130 kg ha^{-1} showed a greater ENA than those treated with that of 90 kg ha^{-1} by 20 m^{-2} ($P = 0.015$) (Table 3.3.8.3 (b)). In Cycle III-C the plots treated with the N rate of 200 kg ha^{-1} showed a greater ENA than those treated with that of 100 kg ha^{-1} by 18 m^{-2} ($P = 0.013$) (Table 3.3.8.3 (b)).

Table 3.3.8.3 (b) ENA (m^{-2}) for N rates at pre-harvest

N rates ($kg\ ha^{-1}$)	*Cycle I*	*Cycle II*	*Cycle III-B*	*Cycle III-C*
0	345	-	350	-
90	-	353	-	-
100	493	-	456	462
130	-	373	-	-
140	491	-	459	-
200	-	-	-	480
P value	< 0.001	= 0.015	< 0.001	= 0.013
L.S.D.	17	16	30	14
CV %	4.6	9.3	8.3	5.9

3.3.8.4 Grain Number per Area (GNA)

Interactions

There was no interaction in GNA between fungicide programmes and N rates in any of the field experiments.

Fungicide Programmes

There was no difference in GNA between fungicide programmes except in Cycle III-C where untreated plots showed a smaller grain number than those treated with fungicides ($P < 0.001$) (Table 3.3.8.4 (a)). The difference ranged from 1600 m^{-2} to 2200 m^{-2} ($P < 0.001$) (Table 3.3.8.4 (a)).

Table 3.3.8.4 (a) GNA for fungicide programmes ($\times 10^4$ m^{-2})

Fungicide Programmes	*Cycle I*	*Cycle II*	*Cycle III-B*	*Cycle III-C*
untrt	1.41	1.62	1.56	1.88
epoxi	1.49	1.66	1.55	2.10
epoxi + kreso	1.41	1.60	-	2.04
epoxi + triflo	1.50	1.65	1.60	2.09
P value	= 0.368	= 0.606	= 0.674	< 0.001
L.S.D.	NS	NS	NS	0.07
CV %	9.8	9.3	9.8	7.0

untrt: untreated; epoxi: epoxiconazole; kreso: kresoxim-methyl; triflo: trifloxystrobin

N rates

Both in Cycle I and Cycle III-B, GNA was the lowest for the plots that received no N fertilizer ($P < 0.001$) (Table 3.3.8.4 (b)). The difference ranged from 7900 m^{-2} to 8600 m^{-2} in Cycle I and it was 7400 m^{-2} in Cycle III-B ($P < 0.001$) (Table 3.3.8.4 (b)). In Cycle II the plots treated with the N rate of 130 kg ha^{-1} showed a greater GNA than those treated with that of 90 kg ha^{-1} by 1300 m^{-1} ($P < 0.001$) (Table 3.3.8.4 (b)). In Cycle III-C the plots treated with the N rate of 200 kg ha^{-1} showed a greater GNA than those treated with that of 100 kg ha^{-1} by 1400 m^{-2} ($P < 0.001$) (Table 3.3.8.4 (b)).

Table 3.3.8.4 (b) GNA for N rates ($\times 10^4$ m^{-2})

N rates (kg ha^{-1})	*Cycle I*	*Cycle II*	*Cycle III-B*	*Cycle III-C*
0	0.90	-	1.08	-
90	-	1.57	-	-
100	1.69	-	1.82	1.96
130	-	1.70	-	-
140	1.76	-	1.82	-
200	-	-	-	2.10
P value	< 0.001	< 0.001	< 0.001	< 0.001
L.S.D.	0.12	0.07	0.13	0.07
CV %	9.8	9.3	9.8	7.0

3.3.8.5 Thousand Grain Weight (TGW)

In the following, TGW of manually-harvested grains is discussed. TGW of combine-harvested grains is given in Appendix 8.

Interactions

There was no interaction in TGW between fungicide programmes and N rates in Cycle I, Cycle II and Cycle III-B. In Cycle III-C, an interaction in TGW was observed between varieties and fungicide programmes. Equinox showed a greater TGW than Hereward with the application of fungicides, but there was no difference in TGW between the two varieties for untreated plots ($P < 0.001$) (Fig. 3.3.8.5). The difference in TGW between the two varieties with the application of fungicides ranged from 3.3 g to 5.6 g ($P < 0.001$) (Fig. 3.3.8.5).

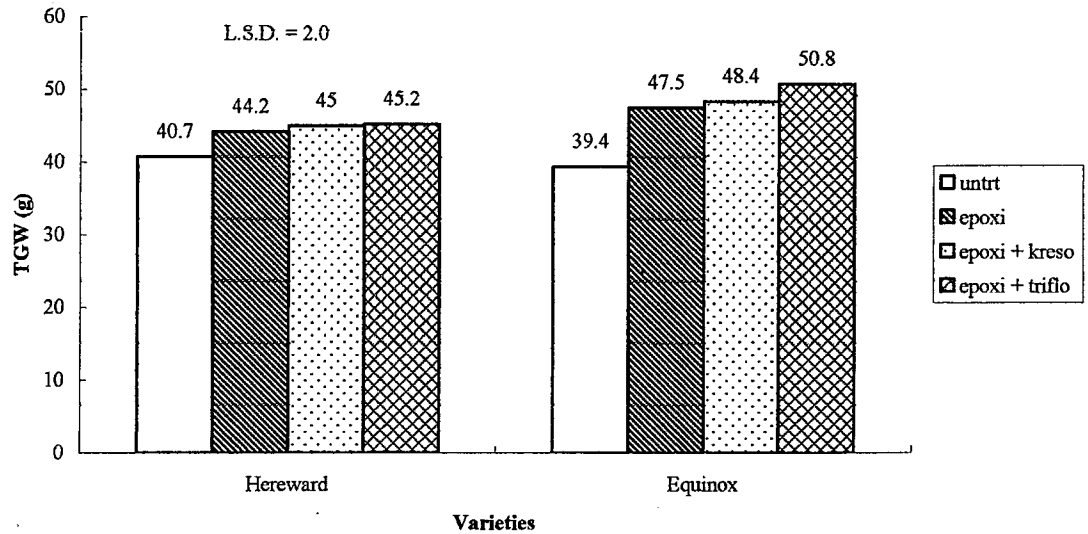


Figure 3.3.8.5 The interaction in TGW (@ 100 %) of combine-harvested grains between varieties and fungicide programmes at harvest in Cycle III-C

untrt: untreated; epoxi: epoxiconazole; kreso: kresoxim-methyl; triflo: trifloxystrobin

Fungicide Programmes

In Cycle I untreated plots showed a smaller TGW compared to those treated with a mixture of epoxiconazole and kresoxim-methyl by 1.5 g and those treated with a mixture of epoxiconazole and trifloxystrobin by 1.8 g, both strobilurin fungicide programmes ($P = 0.031$) (Table 3.3.8.5 (a)). There was no difference in TGW in Cycle II (Table 3.3.8.5 (a)). In Cycle III-B untreated plots showed a smaller TGW than the plots treated with epoxiconazole alone by 1.4 g and those treated with a mixture of epoxiconazole and trifloxystrobin by 2.1 g ($P = 0.003$) (Table 3.3.8.5 (a)), however, the difference between the plots treated with epoxiconazole alone and those treated with a mixture of epoxiconazole and trifloxystrobin was not significant. In Cycle III-C untreated plots showed a smaller TGW than the plots treated with fungicides, with the differences ranging from 5.9 g to 8.0 g ($P < 0.001$) (Table 3.3.8.5 (a)). The plots treated with epoxiconazole alone showed a smaller TGW than those treated with a mixture of epoxiconazole and trifloxystrobin by 2.1 g ($P <$

0.001) (Table 3.3.8.5 (a)).

Table 3.3.8.5 (a) TGW (@ 100 % DM) of manually-harvested grains for fungicide programmes (g)

Fungicide Programmes	*Cycle I*	*Cycle II*	*Cycle III-B*	*Cycle III-C*
untrt	44.5	39.4	44.5	40.0
epoxi	45.6	39.1	45.9	45.9
epoxi + kreso	46.0	39.8	-	46.7
epoxi + triflo	46.3	38.8	46.6	48.0
P value	= 0.031	= 0.393	= 0.003	< 0.001
L.S.D.	1.21	NS	1.11	1.41
CV %	2.7	4.2	2.9	4.4

untrt: untreated; epoxi: epoxiconazole; kreso: kresoxim-methyl; triflo: trifloxystrobin

N rates

In Cycle I the plots treated with the N rate of 140 kg ha^{-1} showed a smaller TGW than those that received no N fertilizer by 1.0 g and those treated with the N rate of 100 kg ha^{-1} by 2.6 g ($P < 0.001$) (Table 3.3.8.5 (b)). In Cycle II there was no difference in TGW between N rates (Table 3.3.8.5 (b)). In Cycle III-B the plots that received no N fertilizer showed a greater TGW than those treated with the N rate of 100 kg ha^{-1} by 1.6 g and those treated with that of 200 kg ha^{-1} by 1.5 g ($P < 0.001$) (Table 3.3.8.5 (b)). In Cycle III-C the plots treated with the N rate of 100 kg ha^{-1} showed a greater TGW than those treated with that of 200 kg ha^{-1} by 1.3 g ($P = 0.011$) (Table 3.3.8.5 (b)).

Table 3.3.8.5 (b) TGW (@ 100 % DM) of manually-harvested grains for N rates (g)

N rates ($kg\ ha^{-1}$)	*Cycle I*	*Cycle II*	*Cycle III-B*	*Cycle III-C*
0	46.8	-	46.7	-
90	-	39.4	-	-
100	45.8	-	45.1	45.8
130	-	39.2	-	-
140	44.2	-	45.2	-
200	-	-	-	44.5
P value	< 0.001	= 0.629	= 0.009	= 0.011
L.S.D.	1.04	-	1.11	1.00
CV %	2.7	4.2	2.9	4.4

3.3.8.6 Harvest Index (HI)

Interactions

No interaction in HI between fungicide programmes and N rates was observed in Cycle I, Cycle II and Cycle III-B. In Cycle III-C, interactions in HI were observed between varieties and fungicide programmes. Equinox showed a greater HI than Hereward with the application fungicides, but there was no difference in HI between the two varieties for untreated plots ($P < 0.001$) (Fig. 3.3.8.6 (a)).

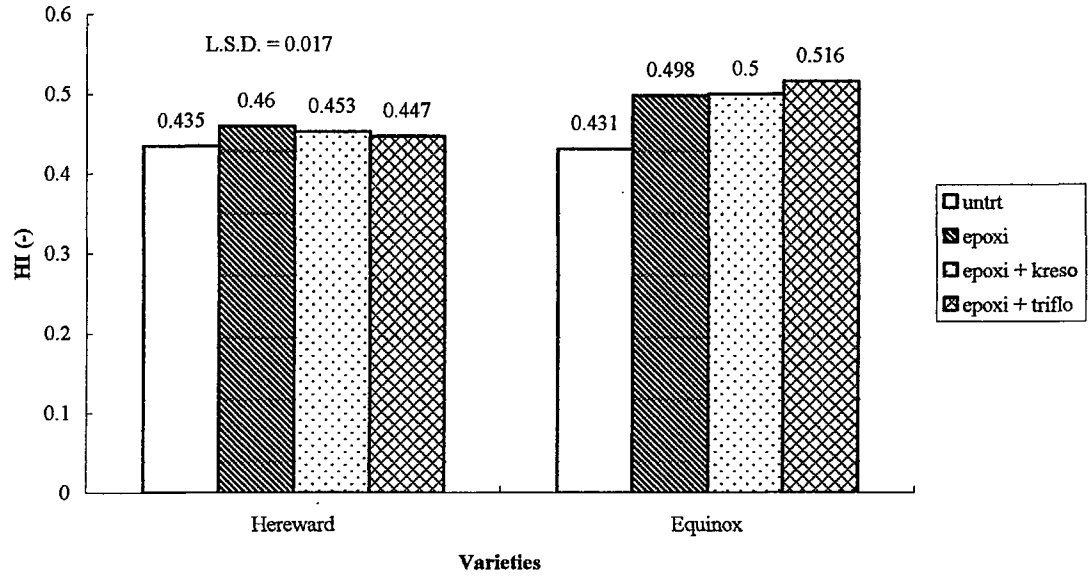


Figure 3.3.8.6 (a) The interaction in HI between varieties and fungicide programmes at pre-harvest in Cycle III-C

untrt: untreated; epoxi: epoxiconazole; kreso: kresoxim-methyl; triflo: trifloxystrobin

Fungicide Programmes

There was no difference in HI between fungicide programmes in Cycle I, Cycle II and Cycle III-B (Table 3.3.8.6 (a)). In Cycle III-C untreated plots showed a smaller HI than those treated with fungicides ($P <$ 0.001) (Table 3.3.8.6 (a)). The difference in HI between untreated plots and those treated with fungicides ranged from 0.044 to 0.049 (Table 3.3.8.6 (a)).

Table 3.3.8.6 (a) HI of manually – harvested crops for fungicide programmes

Fungicide Programmes	*Cycle I*	*Cycle II*	*Cycle III-B*	*Cycle III-C*
untrt	0.385	0.576	0.448	0.433
epoxi	0.385	0.573	0.451	0.479
epoxi + kreso	0.383	0.573	-	0.477
epoxi + triflo	0.390	0.580	0.460	0.482
P value	= 0.390	= 0.159	= 0.326	< 0.001
L.S.D.	NS	NS	NS	0.012
CV %	2.2	1.8	4.3	3.5

untrt: untreated; epoxi: epoxiconazole; kreso: kresoxim-methyl; triflo: trifloxystrobin

N rates

The plots that received no N fertilizer showed a smaller HI compared to those that received N fertilizer in Cycle I ($P < 0.001$) and Cycle III-B ($P < 0.001$) (Table 3.3.8.7 (b)). The difference in HI between the plots that received no N fertilizer and those treated with N fertilizer ranged from 0.051 to 0.055 in Cycle I and from 0.045 to 0.053 in Cycle III-B (Table 3.3.8.7 (b)). The difference in HI between the plots treated with the N rate of 100 kg ha^{-1} and those treated with that of 140 kg ha^{-1} was not significant in these field experiments (Table 3.3.8.7 (b)). In Cycle II, the plots treated with the N rate of 130 kg ha^{-1} showed a greater HI than those treated with that of 90 kg ha^{-1} by 0.005 ($P = 0.018$) (Table 3.3.8.7 (b)). In Cycle III-C, there was no difference in HI between the plots treated with the N rate of 100 kg ha^{-1} and those treated with that of 200 kg ha^{-1} (Table 3.3.8.7 (b)).

Table 3.3.8.6 (b) HI of manually – harvested crops for N rates

N rates ($kg\ ha^{-1}$)	*Cycle I*	*Cycle II*	*Cycle III-B*	*Cycle III-C*
0	0.350	-	0.420	-
90	-	0.573	-	-
100	0.401	-	0.465	0.465
130	-	0.578	-	-
140	0.405	-	0.473	-
200	-	-	-	0.470
P value	< 0.001	= 0.018	< 0.001	= 0.300
L.S.D.	0.007	0.005	0.016	NS
CV %	2.2	1.8	4.3	3.5

3.3.9 The Relationship between Yield Components

3.3.9.1 GNA and Yield

A linear relationship was observed between GNA and grain yield of manually-harvested grains (Fig. 3.3.9.1). Variability in grain yield was mostly attributable to variability in grain number per area (GNA) as shown in Table 3.3.9 (a). In Cycle I and Cycle III-B, as high as 97 – 98 % of the variability in grain yield was accounted for by GNA. In Cycle II and Cycle III-C, 63 % and 72 % of the variability in grain yield was accounted for by GNA respectively. A slightly greater variability was accounted for by GNA when the analysis was conducted for Hereward only by removing other varieties (Table 3.3.9 (b)).

$$GY = a \times GNA + b \qquad \text{(Eq. 3.3.9.1)}$$

GY: Grain Yield ($g\ m^{-2}$)

GNA: Grain Number per Area ($\times 10^4\ m^{-2}$)

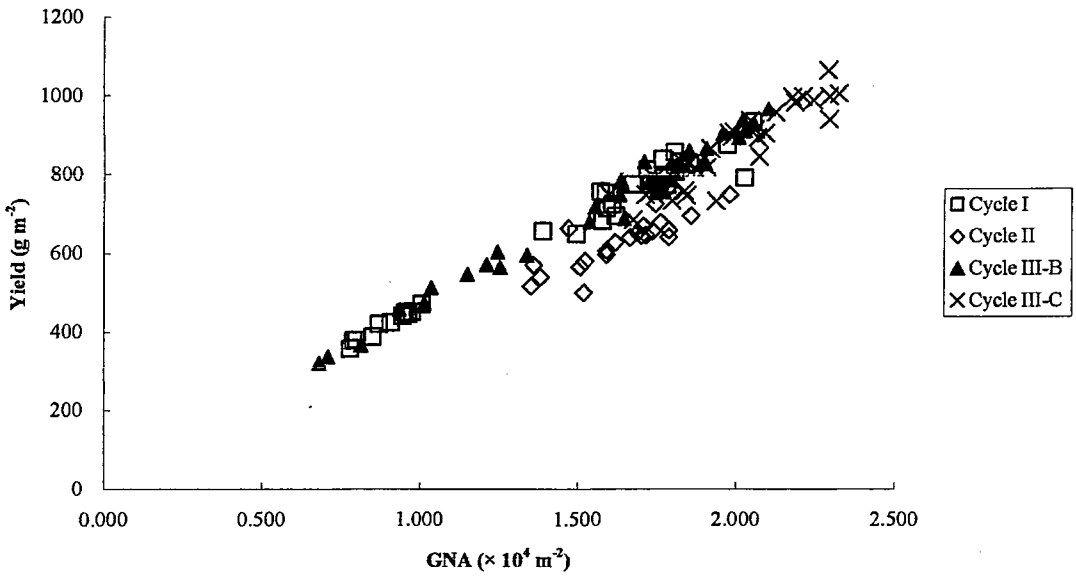


Figure 3.3.9.1 The relationship between GNA and yield (@ 100 % DM) of manually-harvested grains for Hereward at pre-harvest

Table 3.3.9.1 (a) Coefficients of linear regression analysis of the relationship between GNA and grain yield

Field Experiment	*Coefficient (t pr.)*	*Constant (t pr.)*	*P value*	R^2
Cycle I	426 (< 0.001)	40 (= 0.042)	< 0.001	0.97
Cycle II	388 (< 0.001)	NS (= 0.654)	< 0.001	0.63
Cycle III-B	431 (< 0.001)	36 (= 0.043)	< 0.001	0.98
Cycle III-C	610 (< 0.001)	-320 (= 0.002)	< 0.001	0.72

Table 3.3.9.1 (b) Coefficients of linear regression analysis of the relationship between GNA and grain yield for Hereward

Field Experiment	*Coefficient (t pr.)*	*Constant (t pr.)*	*P value*	R^2
Cycle II	337 (< 0.001)	NS (= 0.346)	< 0.001	0.67
Cycle III-C	460 (< 0.001)	NS (= 0.652)	< 0.001	0.82

3.3.9.2 TGW and Yield

In Cycle II, there was no correlation between TGW and grain yield of manually-harvested grains (Fig. 3.3.9.2). TGW and grain yield were negatively correlated in Cycle I and Cycle III-B, while a positive correlation was observed in Cycle III-C (Table 3.3.9.2).

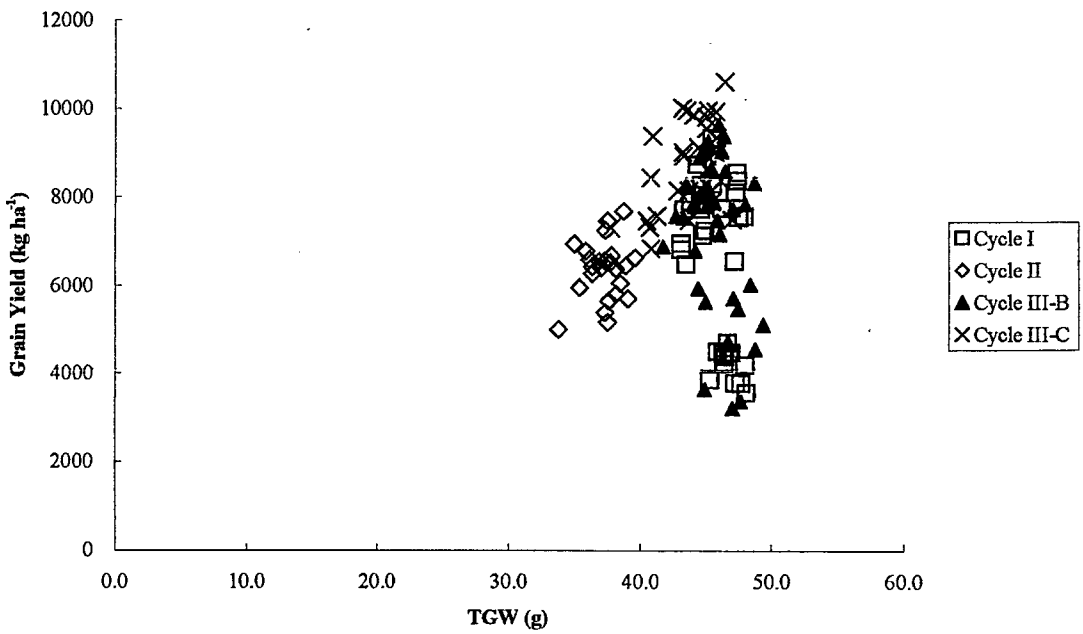


Figure 3.3.9.2 The relationship between TGW and grain yield of manually-harvested grains

Table 3.3.9.2 Pearson's Correlation Analysis between TGW and Yield for Hereward

Field Experiment	*Correlation*	*P value*
Cycle I	-0.408 ($n = 33$)	= 0.018
Cycle II	NS ($n = 24$)	= 0.380
Cycle III-B	-0.343 ($n = 36$)	= 0.040
Cycle III-C	0.480 ($n = 31$)	= 0.006

3.3.10 Aboveground DMW increase rate, Grain DMW increase rate and Vegetative DMW decrease rate

Cycle II

A very high positive correlation was observed between the increase rate of aboveground DMW and that of grain DMW and a very high negative correlation was observed between the increase rate of aboveground DMW (Fig. 3.3.10 (a)) and the decrease rate of vegetative DMW (Fig. 3.3.10 (b)) (Table 3.3.10 (a)). A negative correlation was observed between the increase rate of grain DMW and the decrease rate of vegetative weight, but it was not as high as that observed between the increase rate of aboveground DMW and the decrease rate of vegetative DMW (Fig. 3.3.10 (c)) (Table 3.3.10 (a)).

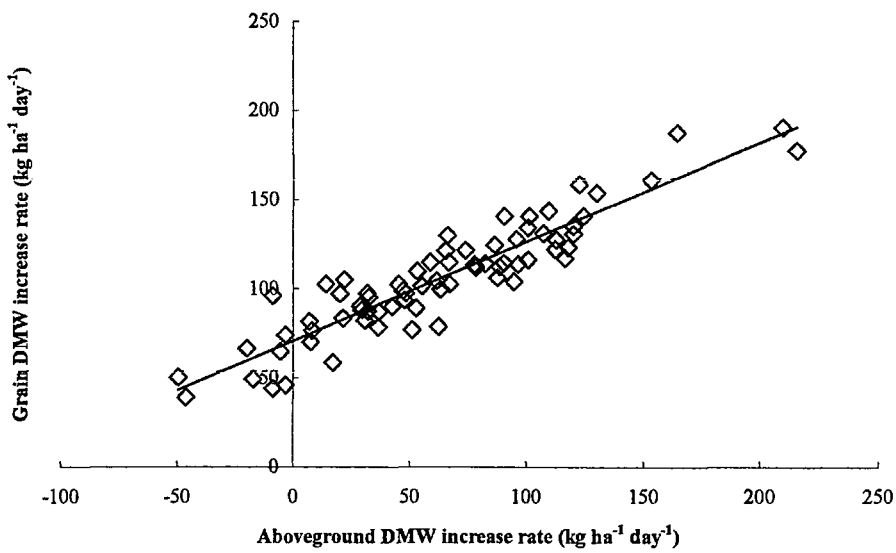


Figure 3.3.10 (a) The relationship between aboveground DMW increase rate and grain DMW increase rate during the latter phase of grain filling in Cycle II

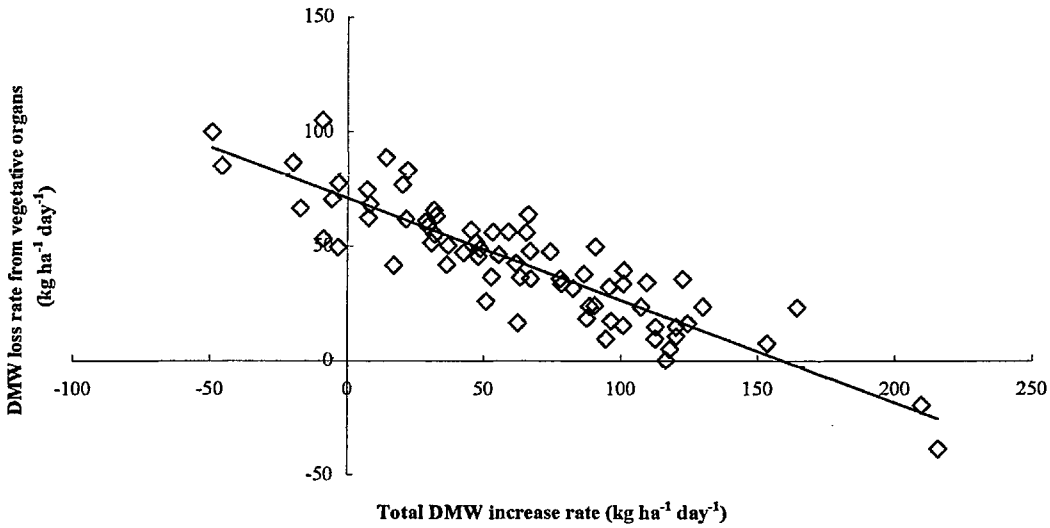


Figure 3.3.10 (b) The relationship between aboveground DMW increase rate and vegetative DMW decrease rate during the latter phase of grain filling in Cycle II

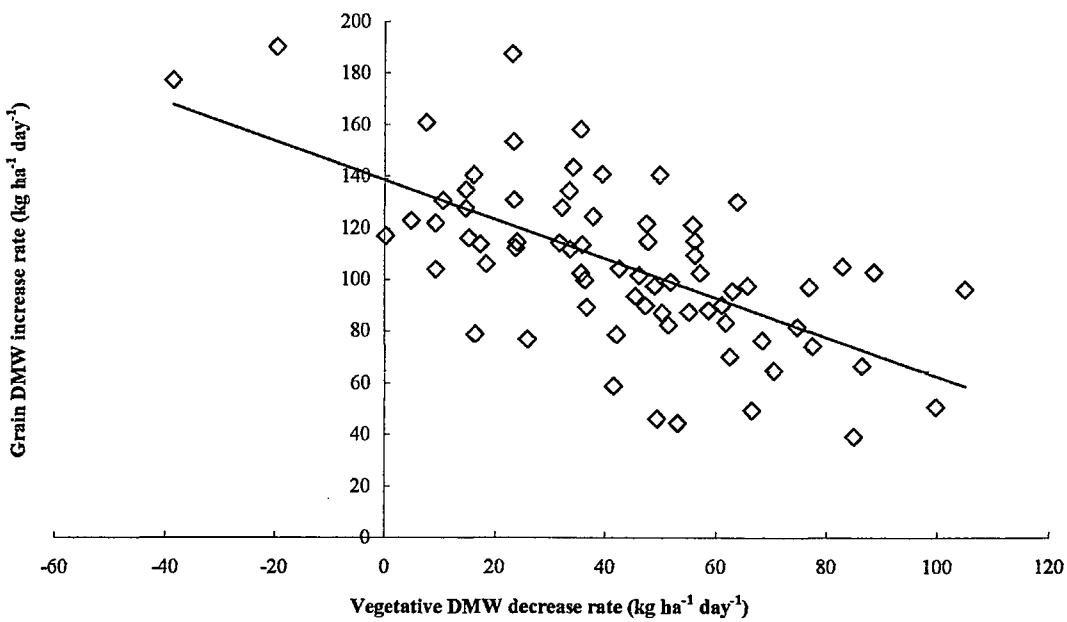


Figure 3.3.10 (c) The relationship between vegetative DMW loss rate and grain DMW increase rate during the latter phase of grain filling in Cycle II

Table 3.3.10 (a) Pearson's Correlation Analysis between aboveground DMW increase rate, grain DMW increase rate and vegetative DMW decrease rate for Hereward between the period from 3 weeks after anthesis to pre-harvest in Cycle II (n = 24)

Element	*Grain DMW Increase rate*	*Vegetative DMW Decrease rate*
Aboveground DMW Increase rate	0.917***	-0.926***
Grain DMW Increase rate	-	-0.698***

*** $P < 0.001$

Cycle III-B

A high positive correlation was observed between the increase rate of aboveground DMW and that of grain DMW (Fig. 3.3.10 (d)), while a high negative correlation was observed between the increase rate of aboveground DMW and the decrease rate of vegetative DMW (Fig. 3.3.10 (e)) (Table 3.3.10 (b)). No correlation was observed between the increase rate of grain DMW and the decrease rate of vegetative DMW (Fig. 3.3.10 (f)).

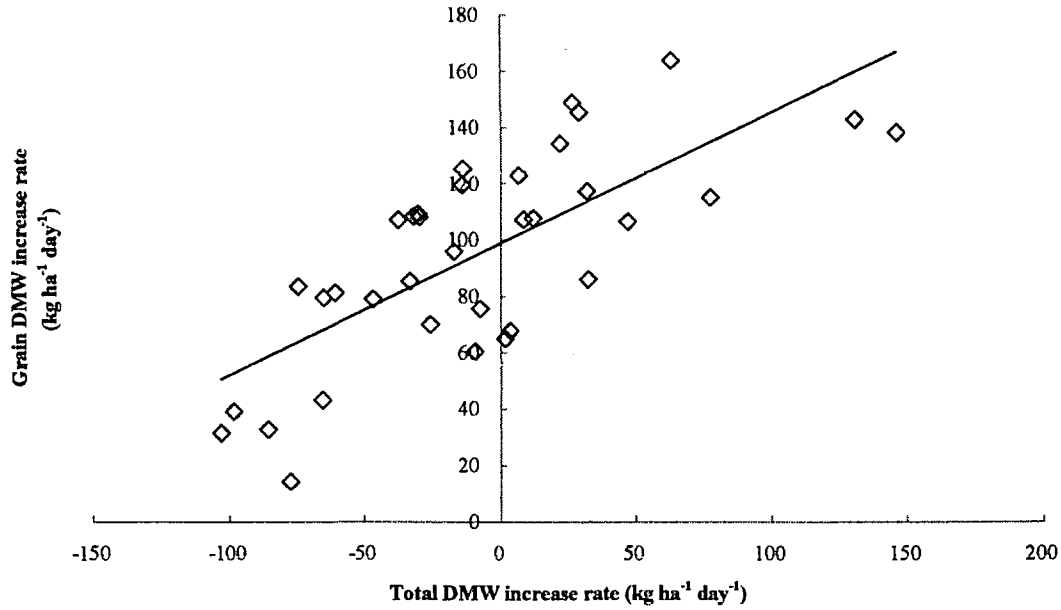


Figure 3.3.10 (d) The relationship between aboveground DMW increase rate and grain DMW increase rate during grain filling period in Cycle III-B

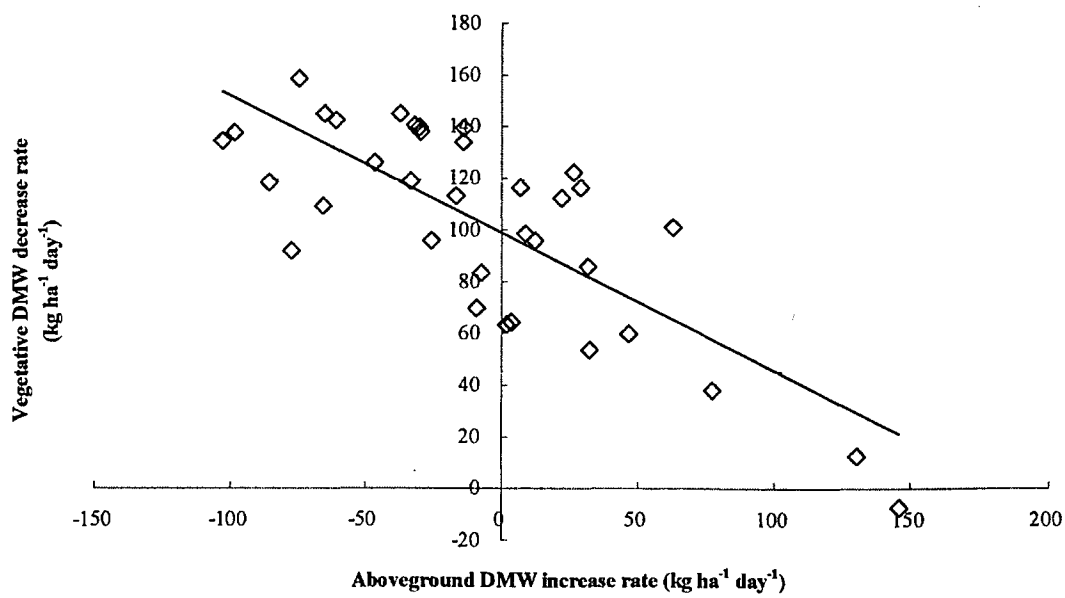


Figure 3.3.10 (e) The relationship between aboveground DMW increase rate and vegetative DMW decrease rate during grain filling in Cycle III-B

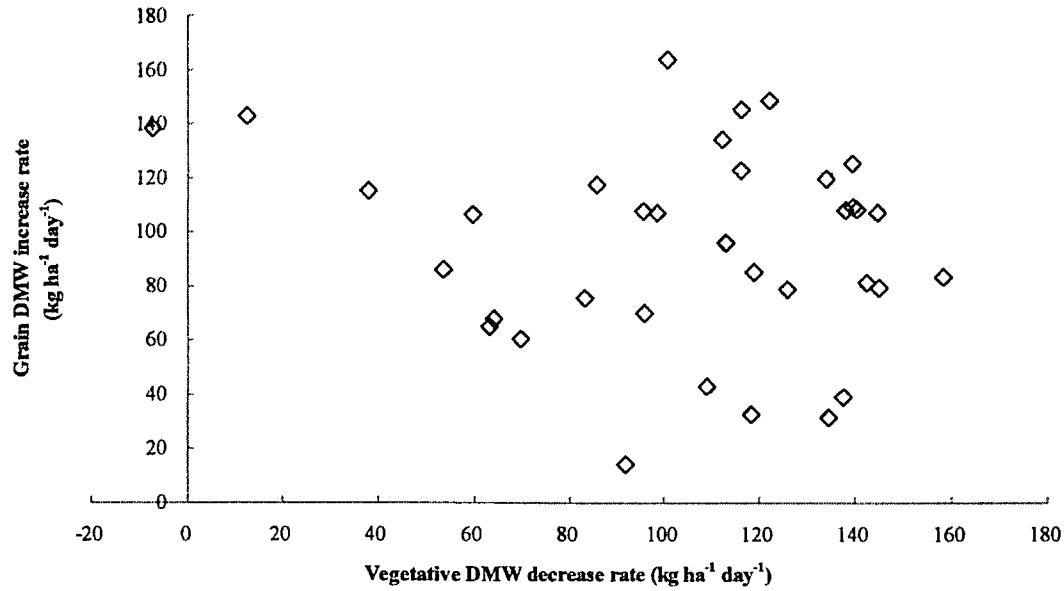


Figure 3.3.10 (f) The relationship between vegetative DMW decrease rate and grain DMW increase rate during grain filling in Cycle III-B

Table 3.3.10 (b) Pearson's Correlation Analysis between aboveground DMW increase rate, grain DMW increase rate and vegetative DMW decrease rate for Hereward between the period from 3 weeks after anthesis to pre-harvest in Cycle III-B (n = 33)

Element	*Grain DMW Increase rate*	*Vegetative DMW Decrease rate*
Aboveground DMW Increase rate	0.738***	-0.780***
Grain DMW Increase rate	-	NS

***$P < 0.001$

3.3.11 Single Grain Dry Matter (SGDM) increase rate

There was no interaction in SGDM increase rate between fungicide programmes and N rates in Cycle III-B.

Fungicide Programmes

The increase rate of single grain DMW was affected by fungicide programmes neither during the early phase of grain filling nor later phase (Table 3.3.11 (a)).

Table 3.3.11 (a) SGDM increase rate for fungicide programmes in Cycle III-B (mg day^{-1})

Period	*untrt*	*epoxi*	*epoxi + triflo*	*P value*	*CV %*
From 269 – 288 DAS To 295 – 302 DAS	1.13	1.20	1.23	= 0.087	9.4
From 295 – 302 DAS To 311 – 323 DAS	0.19	0.23	0.23	= 0.236	32.0

untrt: untreated; epoxi: epoxiconazole; triflo: trifloxystrobin

N rates

The increase rate of single grain DMW was affected by N rates neither early phase of grain filling nor later phase (3.3.11 (b)).

Table 3.3.11 (b) SGDM increase rate for N rates in Cycle III-B (mg day^{-1})

Period	*0 kg N*	*100 kg N*	*140 kg N*	*P value*	*CV %*
From 269 – 288 DAS To 295 – 302 DAS	1.23	1.16	1.17	= 0.283	9.4
From 295 – 302 DAS To 311 – 323 DAS	0.22	0.21	0.21	= 0.964	32.0

kg N: kg N ha^{-1}

3.4 Discussion

3.4.1 Senesced Leaf Area

Both in Cycle II and Cycle III-B where senesced leaf area was assessed, there was no interaction between fungicide programmes and N rates. In Cycle II fungicide programmes hardly affected the percentage of senesced leaf area except on leaf 3 observed at anthesis when the plots treated with a mixture of epoxiconazole and trifloxystrobin showed a smaller percentage of senesced leaf area than untreated plots and those treated with epoxiconazole alone. The situation was very different in Cycle III-B where fungicide programmes caused differences in the percentage of senesced leaf area at and following anthesis. At anthesis untreated plots showed a greater percentage of senesced leaf area than the plots treated with fungicides but there was no difference between the two fungicide programmes, a triazole programme and a mixture of triazole and strobilurin. However, approximately two to three weeks after anthesis, the difference in the percentage of senesced leaf area between the two fungicide programmes was observed, always showing a greater percentage of senesced leaf area for the triazole programme than a mixture of triazole and strobilurin indicating a better performance of a mixture of triazole and strobilurin compared to triazole alone in maintaining green leaf area. This agrees with the observation made by Jones (1998) that the addition of a strobilurin chemistry to epoxiconazole maintained green canopy duration longer than when the crop was treated with epoxiconazole alone.

3.4.2 LAI, GLAD, PAR interception and RUE

Both in Cycle I and Cycle III-B, LAI tended to be greater for the plots treated with fungicides than untreated plots and especially the performance of the plots treated with a mixture of epoxiconazole and trifloxystrobin was good especially at the later part of grain filling. Consequently in Cycle III-B, the plots treated with a mixture of epoxiconazole and trifloxystrobin showed a greater GLAD than untreated plots and those treated with epoxiconazole alone. Delayed senescence of flag leaf has been reported by Gooding *et al.* (2000) and Ruske *et al.* (2001) following the application of azoxystrobin, another strobilurin fungicide. In this study, GLAD significantly accounted for 87 – 88 % of the variability in aboveground DMW and yield at pre-harvest in polynomial regression, which indicates that at a high range of GLAD, an extra increase in GLAD would make less contribution probably due to either mutual shading of high LAI or relatively earlier cessation of grain growth (Davies *et al.*, 1984; Ruske *et al.*, 2001) or both of the two causes.

In Cycle II, fungicide programmes did not affect LAI in any different manner. In this field experiment the severity of *Septoria* diseases was not different either between fungicide programmes. This would imply that there was no physiological greening effect of fungicides in this field experiment. However, great care must be taken to interpret the results of this field experiment. As it has been already mentioned, the crops grown in Cycle II were late-drilled and their size stayed particularly small (Photograph 4) even at the maximum canopy compared to the field experiments conducted in other years.

In Cycle III-B, calculated PAR interception was significantly influenced both by fungicide programmes and

N rates, while there was no interaction between the two. The magnitude of influence was greater of N rates compared to fungicide programmes, for example, the application of a mixture of epoxiconazole and trifloxystrobin caused an increase of PAR interception by 14 % compared to untreated, while an increase of that by 51 % occurred when the N rate of 140 kg ha^{-1} was applied compared to when no N was applied.

Neither fungicide programmes nor N rates significantly affected RUE in this study. N fertilizer is known to increase RUE by about 10 % at most (Biscoe and Gallagher, 1978). The differences in RUE between the plots treated with the N rate of 100 kg ha^{-1} and those treated with no or 140 kg ha^{-1} were nearly 8 – 9 %. This, however, was not found significant.

3.4.3 Dry Matter Accumulation and Partitioning

Overall fungicide programmes did not significantly affect aboveground DMW apart from the untreated plots in Cycle III-C which showed an exceptionally high level of *Septoria* diseases as mentioned earlier. This seems to coincide with Jones *et al.* (2001) that no significant effects of fungicides on crop biomass were observed following the application of fungicides where three fungicide programmes (i.e. epoxiconazole alone, azoxystrobin alone and a mixture of epoxiconazole and kresoxim-methyl) in combination of three dose rates (i.e. quarter, half and full rate) were tested, although there was an indication that the plots treated with a mixture of epoxiconazole and kresoxim-methyl at full rate and those treated with epoxiconazole at half rate showed greater canopies than others.

In Cycle II of this study, a significantly greater aboveground DMW was observed for the plots treated with a mixture of epoxiconazole and kresoxim-methyl when treated with the N rate of 130 kg ha^{-1}. Such an interaction between fungicide programmes and N rates in aboveground DMW was observed only in this field experiment. Cycle II was not a standard field experiment and did not show any significant response to strobilurin fungicides, however, this interaction suggests that there might be some physiological change caused by the application of a mixture of epoxiconazole and kresoxim-methyl, even though it did not relate to grain yield at harvest.

Even though aboveground DM was not found to be responsive to the application of fungicides irrespective of triazole alone or a mixture of strobilurin and triazole, DMW of green leaf tended to be greater for the plots treated with fungicides, especially those treated with a mixture of epoxiconazole and trifloxystrobin. The opposite tendency was observed with DMW of senesced leaf. These observations were more distinct when the percentage of DMW of green leaf and senesced leaf to the aboveground DMW was calculated respectively. A greater percentage of DM was partitioned to green leaf and a smaller percentage of DM was partitioned to senesced leaf respectively for the plots treated with fungicides, especially those treated with a mixture of epoxiconazole and trifloxystrobin. These observations coincide with the observation with green leaf area duration (GLAD) where the plots treated with a mixture of epoxiconazole and trifloxystrobin tended to maintain GLAD longer than untreated plots and those treated with epoxiconazole alone.

3.4.4 Yield and Yield Components

CV % was greater for the yield of manually-harvested grains (data not shown) than that of combine-harvested grains for all the field experiments obviously reflecting the difference in the number of samples taken between the two methods of harvest. Despite the limitation to the number of samples that could be taken in the case of manual harvest, it has the advantage of being able to sample grains of all sizes, while with combine-harvest, smaller grains tend to be lost. Neither in Cycle I, nor Cycle II nor Cycle III-B, any difference in yield of manually-harvested grains caused by different fungicide programmes was observed. In Cycle III-C, untreated plots showed a lower yield of manually-harvested grains probably due to much higher level of *Septoria* diseases observed for untreated plots in this field experiment (Black, 2003). Yield of combine-harvested grains was not significantly affected by fungicide programmes in Cycle I, although numerically the plots treated with a mixture of epoxiconazole and trifloxystrobin showed a greater yield than untreated plots and those treated with other fungicide programmes. In King (2002), only a marginal yield enhancement was reported for the first year wheat as the effect of applying mixtures of triazole and strobilurin, i.e. a mixture of epoxiconazole and azoxystrobin and a mixture of epoxiconazole and trifloxystrobin, over non-strobilurin fungicide programme, i.e. epoxiconazole and morpholine, while for the second year wheat no significant yield difference was observed between fungicide programmes. As to Cycle III-B, yield of combine-harvested grains was greater for the plots treated with a mixture of epoxiconazole and trifloxystrobin than untreated plots and those treated with epoxiconazole alone. This suggests that applying the combination of triazole and strobilurin has advantage to applying triazole alone with respect to yield as reported, for example, by Jones *et al.* (2001) with a mixture of epoxiconazole and

kresoxim-methyl over epoxiconazole alone and by Ruske *et al.* (2001) as the effect of adding azoxystrobin to triazole, however, it was not the case for Hereward in their study. In Cycle III-C, yield of combine-harvested grains was greater for the plots treated with a mixture of epoxiconazole and trifloxystrobin than for those treated with epoxiconazole alone and those treated with a mixture of epoxiconazole and kresoxim-methyl. This agrees well with the observation made in Cycle III-B and implies further that within the two strobilurin fungicide programmes, a mixture of epoxiconazole and trifloxystrobin performed better than a mixture of epoxiconazole and kresoxim-methyl. From different results between the yield of manually-harvested and combine-harvested yield, it could be considered that yield difference between fungicide programmes was so small, even if it exists, that only combine-harvested yield could detect it.

Taking a look at yield components, Ear Number per Area (ENA), Grain Number per Area (GNA) and Harvest Index (HI) were not significantly altered by fungicides except for Cycle III-C where ENA was low for untreated plots. It is considered to be attributable to an exceptionally high level of *Septoria* disease observed with untreated plots in this field experiment as previously mentioned (Black, 2003). Otherwise, this study showed that it is not likely that ENA, GNA and HI are significantly affected by fungicide application. In Cycle III-C, the second fungicide application was delayed to GS 59 due to windy condition which prevailed at the time of the planned fungicide application (GS 39).

TGW was significantly affected by fungicide application except in Cycle II. In Cycle I, Cycle III-B and

Cycle III-C, TGW tended to be lower for untreated plots than for the plots treated with fungicides, which agree with the literature (Simon *et al.*, 2002). A significant difference in TGW between triazole programme and a mixture of triazole and strobilurin programme was observed in Cycle III-C where the plots treated with a mixture of epoxiconazole and trifloxystrobin showed a greater TGW than those treated with epoxiconazole alone. As a similar effect of strobilurin fungicides, Gooding *et al.* (2000) reported an increased mean grain mass following the application of azoxystrobin at or close to flag leaf emergence and ear emergence. The increased yield following the application of strobilurin fungicide programmes reported by King (2002) which has been previously mentioned in this section was mostly attributable to grain size. Cycle III-C was different from Cycle I and Cycle III-B in that Equinox was tested in Cycle III-C. Equinox has been reported to be responsive to kresoxim-methyl to a greater extent than Hereward (Bayles, 1999) and in Cycle III-C, TGW was observed to be greater for Equinox than for Hereward when fungicides were applied. The interesting point to make here is that neither HI nor GNA are likely to be affected by fungicide programmes, while TGW is. An interpretation could be made, for example supposing a simplified example of 1000 grains of a potential sink size of each grain, "a". If untreated plots and the plots treated with fungicides achieved 60 % and 80 % of the potential sink size respectively at harvest, TGW would be "600a" and "800a" respectively. As HI does not differ between the two treatments, the ratio of aboveground DMW of untreated plots to that of the plots treated with fungicides would be 3 : 4 indicating a smaller aboveground DMW of untreated plots in comparison with the plots treated with fungicides. The same reasoning could be applied to the plots treated with triazole and those treated with a mixture of triazole and strobilurin in Cycle III-C. In reality there was no significant difference in aboveground DMW at pre-harvest between fungicide

programmes except for untreated plots of Cycle III-C observed with a particularly severe level of *Septoria* diseases as it has been already mentioned. However, it should be noted that the plots treated with a mixture of epoxiconazole and trifloxystrobin showed numerically a greater aboveground DMW than the plots treated with epoxiconazole alone by 900 kg ha^{-1} where L.S.D. was 1000 kg ha^{-1}. This difference might have been found to be significant if a greater number of replicates were tested or a greater number of samples taken.

3.4.5 The relationship between yield components

Correlation between TGW and yield was weaker than that between GNA and yield. This agrees with the observation by Darwinkel (1983) that grain yield is largely determined by the number of grains produced. Interestingly the manner of correlation between TGW and yield was not very consistent across the field experiments. A negative correlation was observed between the two components in Cycle I and Cycle III-B, while the correlation was positive in Cycle III-C. It might have something to do with the design of N treatments of the field experiments. Both Cycle I and Cycle III-B had the N treatment of untreated, while Cycle III-C did not. TGW tended to be large when no N fertilizer was applied, which might have caused the correlation between TGW and yield to be negative in Cycle I and Cycle III-B in this study.

3.4.6 Aboveground DMW increase rate, Grain DMW increase rate and Vegetative DMW decrease rate

A very high correlation observed between the increase rate of aboveground DM and that of grain DMW during the period between three weeks after anthesis and pre-harvest indicates that the photosynthates

assimilated during this period were utilized almost fully by grains or, in other words, was translocated to grains. On the other hand, the correlation observed in Cycle III-B was lower than that in Cycle II. This could mean that in Cycle III-B the photosynthates assimilated during the later part of grain filling might not have been fully utilized by grains indicating some extent of sink limitation. Another interesting point to be mentioned as to the difference between Cycle II and Cycle III-B is the correlation between the increase rate of grain DMW and the decrease rate of vegetative DMW. In Cycle II there was a medium correlation between the two, while in Cycle III-B there was no significant correlation. Despite that the decrease rate of vegetative DMW does not discriminate between the assimilates produced during grain filling period and those stored during vegetative growth, it seems that in Cycle II stored assimilates made more contribution to grain growth than in Cycle III-B. It is known that under stressed conditions such as drought, grains tend to utilize a greater portion of stored assimilates (Wardlaw, 1967) than usual years when they almost solely depend on assimilates produced by flag leaf during grain filling period (Evans *et al.*, 1975).

Chapter 4

Nitrogen Accumulation and its Relation to DM Accumulation

4.1 Introduction

4.1.1 N in Wheat Crops

N is the most limiting nutrient in crop production and therefore, has been an area of intensive investigation by agronomists and plant physiologists (Novoa and Loomis, 1981). N functions in a number of ways in wheat plants during the growing season. Biological functions of N in wheat are similar to other C3 plants, the most important role of which is as components of photosynthesis apparatus. As much as 75 % of the total reduced N in a leaf is considered to be associated with photosynthesis mainly in two forms, soluble proteins involved in the dark reactions and chloroplast-related compounds involved in the light reactions (Field and Mooney, 1986). Ribulose 1,5-bisphosphate carboxylase alone accounts for up to 50 % of the soluble protein in C_3 leaves (Sinclair and Horie, 1989). The importance of N in wheat is not only limited to its functional roles but also is extended to roles in determining the quality of harvested grains. For example, in the case of bread-making wheat, crude protein concentration is one of the key features in order to sell grains at premium prices in the market and it should fall between 11 and 13 % at 14 % moisture content (Gooding and Davies, 1997). Obviously, requirements of crude protein concentration vary for different product uses. Wheat for biscuits, noodles and feed requires less grain N concentration compared to wheat for bread and pasta. Cereal protein content could be important from the perspective of human needs for

dietary protein in situations where legume and animal proteins are lacking.

4.1.2 Effects of N on Light Interception and Radiation Use Efficiency

As has been already discussed in Chapter 3, DM accumulation is determined by light interception and radiation use efficiency (RUE). The main components that determine light interception of a given canopy are its size (LAI) and structure (leaf angle). LAI increases through leaf expansion and decreases through leaf senescence. Both processes are greatly affected by the quantity of N available to the canopy (Gimenez *et al.*, 1994). Among factors that influence RUE, species and leaf CO_2 exchange rate are considered to be of particular importance in causing variation in the RUE (Sinclair and Muchow, 1999). C_4 species tend to show greater RUE compared to C_3 species (Murata, 1981) probably due to different photosynthesis pathways between the two species. Variation in the RUE, however, exists within C_3 varieties and is considered to be caused by differences in the energy content of biochemical constituents of the plant products (Sinclair and Muchow, 1999) implying that proteins and lipids may be produced at the cost of carbohydrate, i.e. yield. RUE is influenced to a great extent by CO_2 exchange rate and, therefore, is sensitive to the factors that could affect CO_2 exchange rate (Sinclair and Muchow, 1999). As great proportion of leaf N is associated with photosynthesis apparatus as has been discussed in the section of 4.1.1, it is not surprising to find a close correlation between leaf N and leaf CO_2 exchange rate (Evans, 1983; Evans, 1989). Sinclair and Horie (1989) observed a relationship of positive curvilinear function between leaf N content per unit area and RUE for maize, rice and soybean and modelled RUE for each crop based on the relationship between leaf N and photosynthesis rate. They plotted simulated RUE as a function of leaf N content per unit area and suggested

that RUE could become very sensitive to leaf N content per unit area for situations where leaf N content per unit area is in the lower range. It should be noted that leaf N content per unit area is translated to be leaf N concentration on a leaf area basis. Thus, RUE is affected by leaf N concentration rather than the total quantity of N available to the canopy (Gimenez *et al.*, 1994).

4.1.3 N Partitioning in Canopy

As leaf N content is closely associated with leaf photosynthetic ability, it is expected that N would be distributed in a given plant canopy in such a way that would maximize canopy photosynthesis, in other words, more N would be partitioned at and close to the upper part of the canopy, while less N at and close to the lower part of the canopy, creating a decreasing gradient of N from the top to the bottom of the canopy (Hirose and Werger, 1987). The main factors which have been considered to control partitioning of leaf N in a dense vegetative canopy are light gradient in the canopy (Hirose *et al.*, 1988) and leaf age (Field, 1983), between which light gradient is regarded to have a more significant effect than leaf age (Hikosaka *et al.*, 1994). Dreccer (1999), however, proposed that light may not be the only regulatory factor that controls the distribution of leaf N by mentioning the cases where no effect of plant density was found on the steepness of leaf N gradients (Sadras *et al.*, 1993; Shiraiwa and Sinclair, 1993). It was suggested that a greater fraction of N tends to be kept in the lower part of the canopy when more N is available in the canopy (Anten, 1995). In comparison between two theoretical genotypes which differ only in the pattern of leaf N distribution during grain filling period with the same total quantity of N in leaves being assumed, Dreccer (1999) argued that the genotype which distributes N in steep manner would be suitable for bread wheat as both maximum

grain yield and maximum protein yield would be obtained, while the genotype which keeps a uniform N distribution would be preferred in barley breeding that aims at high yield with low protein concentration. This method of thinking could be useful in understanding the effects of fungicides and N application on canopy architecture in relation to DM accumulation and N accumulation in grain. In Diagram 4.1.3, some theoretical patterns of changes in leaf N during grain filling period with depth of the canopy are considered. For simplification, the pattern A and the pattern B as well as the pattern C and the pattern D are assumed to be exactly the same at anthesis. The difference between the pattern A and the pattern B lies in the rate of leaf N loss especially from the lower part of the canopy. This situation could be expected for the crops that either do or do not receive fungicides. Both the pattern C and the pattern D show a similar N distribution pattern throughout grain filling period, but the total amount of leaf N in the canopy is different. This might be the case when different rates of N were applied. This is, of course, a too simplified view. In reality both cases are considered to exist in a mix of different proportions. The issue will be further complicated when the effects of environmental factors as well as management factors on N uptake and N translocation are considered.

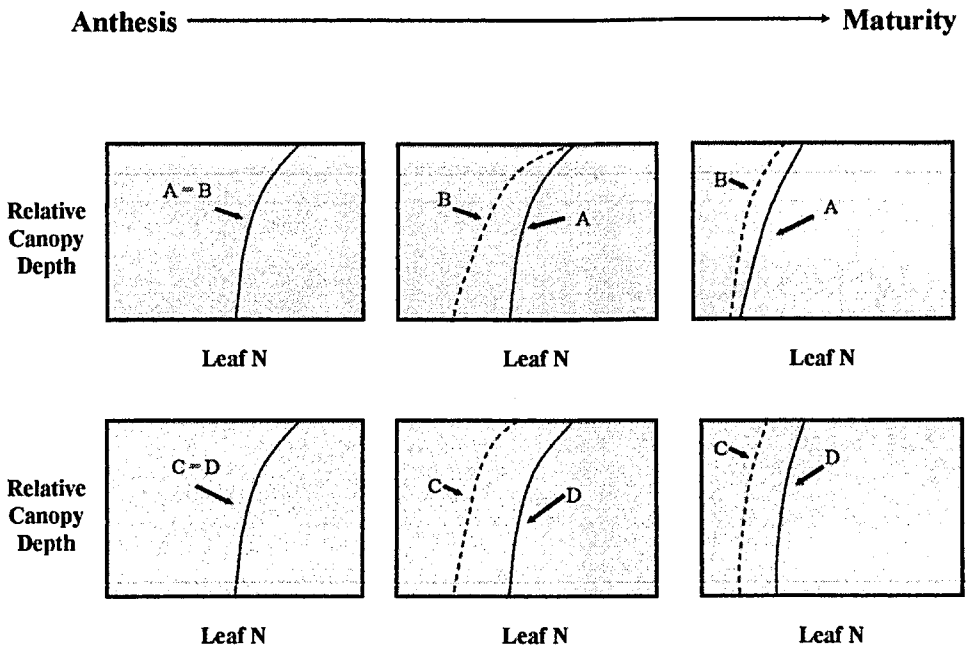


Diagram 4.1.3 Changes in leaf N with depth of the canopy from anthesis to maturity

4.1.4 Plant N Demand and Uptake

Agronomists and plant physiologists have long attempted to elucidate quantitatively the mechanism involved in how plants demand N as well as how they obtain N eventually. Sinclair (1998) pointed out that in order to increase harvest index, much more N is required considering that grain N concentration is more than five times that in straw. The drastic increase in harvest index throughout the latter half of 20th century (Austin *et al.*, 1980; Slafer *et al.*, 1994), therefore, was possible due to ability of the plant to accumulate additional N (Sinclair, 1998). Grain N requirement is met mostly by redistribution of the N which is accumulated in stem and leaves prior to anthesis (Austin and Nair, 1963; Austin *et al.*, 1977; Spiertz and De Vos, 1983; Vouillot and Devienne-Barret, 1999). Genetic variability with respect to N accumulation and remobilization, however, has been demonstrated (Johnson *et al.*, 1967; Van Sanford and MacKown, 1987). For example, Austin *et al.* (1977) reported a high grain-protein genotype of wheat, Atlas 66, which took up approximately 119 kg ha^{-1} of N during grain filling compared with the N uptake of 35 kg ha^{-1}, the average value of other genotypes they tested.

Environmental conditions are known to play major roles in determining the relative contribution of N uptake after anthesis to grain N accumulation (McDonald, 1989). Under favourable soil water (Harper *et al.*, 1987) and soil N conditions (Peterson *et al.*, 1975), N uptake after anthesis has been observed. Elevated temperature beyond 30 ^{0}C tends to reduce grain carbohydrate accumulation but grain N accumulation appears relatively unaffected (Jenner *et al.*, 1991). Grain N accumulation is controlled by the source factor to a greater extent than carbohydrate accumulation (Borghi *et al.*, 1986; Perez *et al.*, 1989). N is not only taken

up by plants but also is 'lost' from plants (Papakosta and Gagianas, 1991; Raun and Johnson, 1999). Cereal plants release N from plant tissue, predominantly as NH_3 following anthesis (Harper *et al.*, 1987).

4.1.5 Leaf Senescence and Stay Greens

The progress of senescence is usually visually recognized as loss of chlorophyll (Thomas and Howarth, 2000) and is defined as the series of events concerned with cellular disassembly in the leaf and the mobilization of materials released during this process (Thomas and Stoddart, 1980). The onset of senescence can be induced by internal factors such as age as well as external factors. Premature senescence can be initiated by environmental stresses such as temperature, drought, poor light condition, lack of nutrients especially N and pathogen attack (Thomas and Stoddart, 1980; Saulescu *et al.*, 2001).

Ottman and Welch (1988) showed that artificially supplemented photosynthetic photon flux density (PPFD) applied to the lower part of the field-grown maize canopy can delay senescence. Consequently they observed an increased plant N accumulation by as much as 90 % and an increased DM accumulation by 54 %. Their study suggested that leaf senescence is strongly induced by lack of light and at the same time that the genetic potential of maize to produce high yields is not fully exploited in current production systems. Other workers have also proposed that delaying basal leaf senescence would increase carbohydrate production and consequently crop yield (Rousseaux *et al.*, 1997). The implication of these findings is significant.

Attempts have been made to delay leaf senescence, in other words, to maintain green leaf in various ways.

Thomas and Howarth (2000) categorized stay-greens into five functional types, however, they pointed out that, in practice, particular stay-greens may be combinations of two or more functional types. Two types were designated to the senescence where maintenance of chlorophyll is only superficial with physiological function being lost. The other two types maintain greenness either by delaying the initiation of senescence or reducing the rate of senescence. The last type is characterized by enhanced greenness. Although their argument was focused on genetic variations in stay-greens features, it could be applied to stay-greens created by other methods such as chemical application (Schistad and Nissen, 1984).

The merits of stay-greens are very often found as an increased yield especially in the case of fungicide application. However, it is difficult to completely exclude the effects of fungicide application on yield via reduction in foliar diseases from those via stay-greens (Kettlewell *et al.*, 1982; Gooding *et al.*, 1988; Gooding *et al.*, 1994; Gooding *et al.*, 2000). Stay-green hybrids of sorghum showed a significant yield advantage under drought condition during grain filling period by producing 47 % more biomass between anthesis and maturity than hybrids of no stay-green traits under postanthesis drought (Borrell *et al.*, 2000).

4.1.6 Grain N and Bread-Making

In 1987 only 18 % of UK wheat contained protein at or above 11.5 %, the minimum value required for bread-making wheat and UK millers had to purchase wheat throughout the world in order to fill this requirement as well as others (Ford, 1987). Increasing protein concentration of wheat grain has been one of the primary objectives in wheat research. Not to mention much effort made in breeding worldwide (Austin

et al., 1977; McNeal *et al.*, 1978; Stoddard and Marshall, 1990; McKendry *et al.*, 1995), foliar urea application at around the time of anthesis is one of the attempts used to increase grain N concentration (Finney *et al.*, 1957; Powlson *et al.*, 1987; Gooding *et al.*, 1991; Woolfolk *et al.*, 2002).

Nelson-Smith (1990) mentioned the successful harvest of excellent quality in 1989 and pointed out that UK wheat started gaining credit in the world market. Sylvester-Bradley (1990), however, argued that most crops grown for bread-making markets receive more N than recommended rates that are considered to be needed to realize optimum yield. He doubted whether application of 'extra N', no matter whether it is applied as ammonium nitrate or foliar urea spray, would be justifiable. Excess N application of little benefit is not desirable in economic terms as well as from an environmental point of view.

4.1.7 Grain N versus Grain Yield

Unfortunately for growers and millers, it is a well known fact that a negative relationship is often observed between grain N concentration and grain yield (Cox *et al.*, 1985; Simmonds, 1995). According to Penning de Vries *et al.* (1974), 1 g of glucose assimilated by photosynthesis could be converted into 0.83 g of carbohydrate or 0.40 g of protein. As an increase both in grain yield and grain protein comes from photosynthates, a possible competition for limited assimilates between carbohydrate synthesis and protein synthesis might be expected (Austin *et al.*, 1977). Stoddard and Marshall (1990) discussed that it might be the case under the conditions where energy is the limiting factor, however, they argued that such a situation is unlikely to happen often in reality where both protein and biomass production are limited by available

nutrients or water rather than available energy. In a field experiment where increased N uptake and assimilation during grain filling was created by N application at heading, plant carbohydrate reserves were reduced transiently, however, they were then more than compensated for by increased photosynthesis rate and prolonged leaf area duration following the additional N application (Bänziger *et al.*, 1994).

Apart from the energy constraints theory, a cause of the negative relationship is often sought for in the fact that the major part of yield increase in breeding is due to increased harvest index accompanied by little or no increase in biomass production (Day *et al.*, 1985). Increased harvest index via breeding would make the vegetative part of wheat plant smaller and proportionately would decrease available N for translocation to grain. McNeal *et al.* (1972) found a close positive correlation between plant N content and grain N content across wheats of different genetic composition, which indicates a rather constant N harvest index. They thus hypothesized that grain N concentration would be dependent on the amount of carbohydrate accumulated in grain. Much of the variation in grain protein concentration in wheat, however, is considered to be environmental rather than genetic in origin (Stoddard and Marshall, 1990; Smith and Gooding, 1996). A dilution of grain N is often observed, for example, when yield is increased by nutrients other than N or higher seeding rates (Terman *et al.*, 1969).

4.1.8 Effects of Fungicides on Grain N concentration

In the majority of experiments out of 6 field experiments conducted in Shropshire and Gloucestershire in the UK between 1984 and 1990, fungicide application improved both grain N accumulation and grain DM

accumulation to a similar proportion, while DM accumulation was favoured relative to N accumulation in a few exceptions (Gooding *et al.*, 1988). The contrasting effects of fungicides on grain N concentration were attributed to different dominant diseases controlled in different seasons and sites (Gooding *et al.*, 1988). Dimmock and Gooding (2002) deduced from 14 separate experiments conducted at three sites in England between 1983 and 2000 that grain protein concentration is reduced by 0.4 % DM basis for every 7 days of extra maintenance of flag leaf although a large degree of variation existed. At the same time, they observed that mean grain weight was increased in association with a delay in flag leaf senescence following fungicide use with 39 % of the variation accounted for. Effects of fungicide use were not limited to an increase in mean grain weight but extended to an increase in grain N content. Nonetheless these observations seem to agree, at least partly, with the hypothesis of McNeal *et al.* (1972) that grain N concentration is dependent on the amount of carbohydrate accumulated in grain.

4.1.9 N in Soils and its implication on Environment

In most soils, large amounts of N usually as organic forms are found in association with the clay fraction (Newbould, 1989). The soil microbial populations mediate the transition between organic pool and inorganic pool immobilization and mineralization (Powlson, 1993). Environmental conditions such as temperature and moisture are known to affect the rate of both processes. As to N loss, nitrate leaching and emission of nitrous oxide through denitrification have been debated to be a major problem from the view of environment, although ammonia volatilization would be added when livestock is considered. Nitrate and nitrous oxide are known to contribute to eutrophication of water bodies and global warming respectively

(Powslon, 1993). In 1991 Europe adopted an environmental measure called 'the Nitrates Directive (91/676/EC)' to reduce water pollution by nitrate from agricultural sources and to prevent it in the future (Anon., 2002a). According to the Nitrates Directive, waters which contain or could contain, if preventative action is not taken, nitrate concentrations greater than 50 mg litre^{-1} are defined to be polluted waters and all land draining into these polluted waters are designated as Nitrate Vulnerable Zones (NVZs) (Anon., 2002b). There is no doubt that the way of farming would have to be changed by this legislation especially in terms of N fertilization so as to reduce nitrate leaching to the environment.

4.1.10 N Fertilization

A great number of studies were and have been carried out to test the effects of timings and rates of N fertilizer application on wheat (Watson, 1936; Kirda *et al.*, 2001) as well as on many other crops (Errebhi *et al.*, 1998; Raun and Johnson, 1999; Waddell *et al.*, 1999; Grigg *et al.*, 2000; Vetsch and Randall, 2004) to seek for the way to maximize the recovery of applied N. Soil moisture condition is known to affect the recovery of N in the crop (Campbell *et al.*, 1977) and therefore, it is very difficult or even not possible to separate the effects of fertilizer N from those of soil moisture condition on the N recovery of the crop under field condition. Smith and Whitfield (1990), using ^{15}N isotope, tested the effects of top-dressing N with and without irrigation on the recovery of fertilizer N and observed the recovery of top-dressed fertilizer N in crop biomass in the range of 45 – 52 % irrespective of the three application timings, at tillering, early-boot and heading except in an treatment where N was applied at heading in the absence of irrigation, which showed 18 – 26 % less than the average value for all other treatments. The recovery of N fertilizer is namely the

efficiency of N fertilizer use which is one of the major factors to be considered in wheat production (Fischer *et al.*, 1993) to maximize the profit as well as minimize the impact on the environment.

The motivation of increasing the efficiency of use of N fertilizer used to aim at increasing agricultural production. However, concerns over the environmental effects of leached N added another aim that is to minimize N loss from agricultural land (Powlson, 1993). Nitrate leaching from agricultural sources to ground and surface waters has been a worldwide concern (Webb *et al.*, 2000). In Northern Europe, delaying N application until spring is a common practice and generally a higher yield response is found when compared to N application in autumn (Ellen and Spiertz, 1980). This is most likely due to losses of fertilizer N from leaching or denitrification (Craswell and Godwin, 1984), as leaching and denitrification tend to occur during winter in North West Europe when it is cold and wet. The way as to fertilizer N affects the process of leaching, however, is not straightforward. Macdonald *et al.* (1989) indicated that almost all of the nitrate at leaching risk over winter is originated from mineralized organic N, not from unused fertilizer. Therefore, cutting down the fertilizer N would not immediately lead to a great reduction in leaching. The level of organic N in soil needs to decline to achieve substantial reductions in leaching (Davies and Sylvester-Bradley, 1995). Apart from the recovery or efficiency issue, it is prerequisite for growers as well as agronomists to be able to imagine the consequence of applying fertilizer N at a given timing and rate on the biomass production and plant N balance of the crop. Late N application, for example around the time of anthesis, is likely to affect grain N concentration with no or little effect on grain yield (Finney *et al.*, 1957; Spiertz and De Vos, 1983).

4.1.11 Objectives and Hypothesis of Chapter 4

This chapter deals with the Objective (III) in the section of '*1.2 Aim, Objectives and Approach*' of Chapter 1 (page 6), i.e., "to understand the effects of the use of strobilurin fungicides in regard of fertilizer N rates on N accumulation and its relation to DM accumulation of winter wheat", two null hypothesis were set.

Firstly this chapter will test the hypothesis that there is no synergistic effect between the use of strobilurin fungicides and fertilizer N rates on N accumulation and its relation to DM accumulation. Secondly it will test the hypothesis that the use of a strobilurin fungicide in a disease control programme in wheat does not affect N accumulation and its relation to DM accumulation.

Four field experiments under a factorial design of fungicide programmes and N rates as factors were performed. The fungicide programmes included the use of the triazole epoxiconazole alone, and in mixture with either kresoxim-methyl or trifloxystrobin, which are strobilurins. A range of N rates were used which varied according to the specific requirements for each field experiment. Variety was added as an extra factor in two of the four field experiments.

4.2 Materials and Methods

4.2.1 General

The data sets from Cycle I, Cycle II, Cycle III-B and Cycle III-C were used in this chapter. Total N was analyzed in the method described in Chapter 1 for the plant materials sampled for DMW determination in Chapter 3. Some plant parts were mixed for total N determination where enough samples were not available for it as presented in Appendix 5.

4.2.2 Terms and Definitions

The following gives explanation to the methods of data collection and data derivation as well as some terms in the context of this particular study that have not been fully explained in Chapter 1 'General Materials and Methods'.

4.2.2.1 Specific Leaf N

Specific Leaf N (SLN) was defined as the quantity of N (mmol) per unit leaf area (m^{-2}). SLN was calculated for the purpose of evaluating any possible differences in leaf photosynthetic rates between treatments. The data set obtained from Cycle III-B at 237 – 244 DAS (GS 49 – 52) was the only data set that could be used for this purpose. This is due to the fact that N analysis of leaf samples was conducted for a given leaf layer without discriminating green leaf area from senesced leaf area.

4.2.2.2 Apparent Recovery of Fertilizer N

Apparent recovery of fertilizer N is sometimes employed (McDonald, 1989) to evaluate how much fertilizer has been recovered in the crop. Aboveground N content in the crop that received no N fertilizer is regarded as the N that was available to the crop from various sources other than fertilizer during the growing season (Eq. 4.2.2.2). This approach is, of course, a very crude one and is subjected to errors from many sources. For example, mineralization rate of soil organic matter might change when fertilizer is applied and the pattern of root growth of the crop is likely to be influenced by the presence of fertilizer in the soil. Irrespective of its many drawbacks, however, this approach is attractive for ease of its use especially when the use of an isotope tracer to observe the behavior of applied fertilizer is not available.

(Apparent recovery of fertilizer N in the aboveground DM)

$$= \frac{\{(\text{aboveground N}) - (\text{aboveground N in zero N treatment})\} \times 100}{(\text{Fertilizer N applied})} \qquad \text{(Eq.4.2.2.2)}$$

4.2.2.3 Nitrogen Use Efficiency (NUE)

Nitrogen Use Efficiency (NUE) expresses the amount of DM produced per unit of N (Eq. 4.2.2.3 (a)). This is, however, basically the inverse of aboveground N concentration as calculated below by substituting (Aboveground DM weight) of (Eq. 4.3.4.3 (a)) for (Eq. 4.2.2.3 (b)). The result is (Eq.4.2.2.3 (c)).

(NUE) = (Aboveground DMW)/(Aboveground N content) (Eq. 4.2.2.3 (a))

(Aboveground N concentration) = {(Aboveground N content)×100}/(Aboveground DMW)

This can be written as

(Aboveground DMW) = {(Aboveground N content)×100}/(Aboveground N concentration)

(Eq. 4.2.2.3 (b))

(NUE) = 100/(Aboveground N concentration) (Eq. 4.2.2.3 (c))

4.3 Results

4.3.1 N content

4.3.1.1 Aboveground N content

There were no interactions in aboveground N content at pre-harvest between fungicide programmes and N rates in any of the field experiments.

Fungicide Programmes

No difference in aboveground N content at pre-harvest was observed between fungicide programmes in Cycle I, Cycle II and Cycle III-B (Table 4.3.1.1). In Cycle III-C, untreated plots had a significantly lower aboveground N content at pre-harvest than the plots treated with fungicides with the difference ranging from 34 to 40 kg ha^{-1} ($P < 0.001$) (Table 4.3.1.1).

Table 4.3.1.1 Aboveground N content at pre-harvest for fungicide programmes (kg ha^{-1})

Fungicide Programmes	*Cycle I**	*Cycle II*	*Cycle III-B*	*Cycle III-C*
untrt	188	184	222	294
epoxi	188	189	227	329
epoxi + kreso	175	185	-	328
epoxi + triflo	187	181	232	334
P value	= 0.295	= 0.755	= 0.584	< 0.001
L.S.D.	NS	NS	NS	20
CV %	9.2	11.3	10.4	8.7

untrt: untreated; epoxi: epoxiconazole: kreso: kresoxim-methyl; triflo: trifloxystrobin

*data from 6th sampling (284 – 286 DAS)

N rates

In all the four field experiments, the greater the N rate, the greater the aboveground N content at pre-harvest. The differences in aboveground N content at pre-harvest between N rates were all significant for each field experiment at the 0.1 % level. The relationship between fertilizer N rate and aboveground N content at pre-harvest is shown in Figure 4.3.1.1 (a) for each field experiment. Only two N rates were tested in Cycle II and Cycle III-C and therefore it was not possible to judge whether increasing N rate beyond the higher N rate tested would have increased the crops' N content by the crop any further. In Cycle I, aboveground N content did not appear to have reached a plateau at the highest N rate tested (140 kg ha^{-1}), while in Cycle III-B, there was some evidence that it may have at 140 kg ha^{-1}. The change in aboveground N in time course is found in Figure 4.3.1.1 (b).

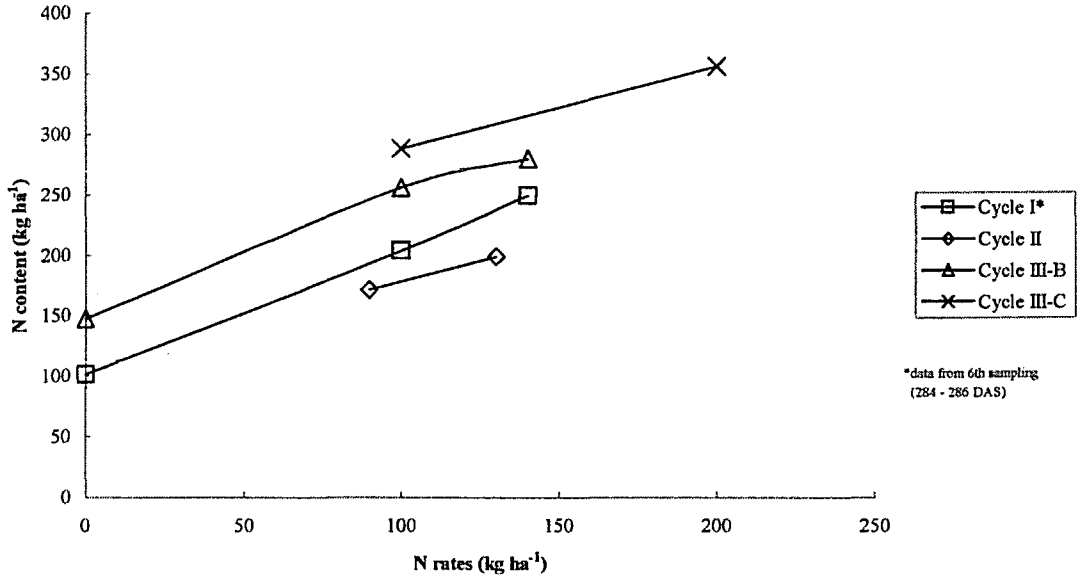


Figure 4.3.1.1 (a) The response of aboveground N content to N rates at pre-harvest

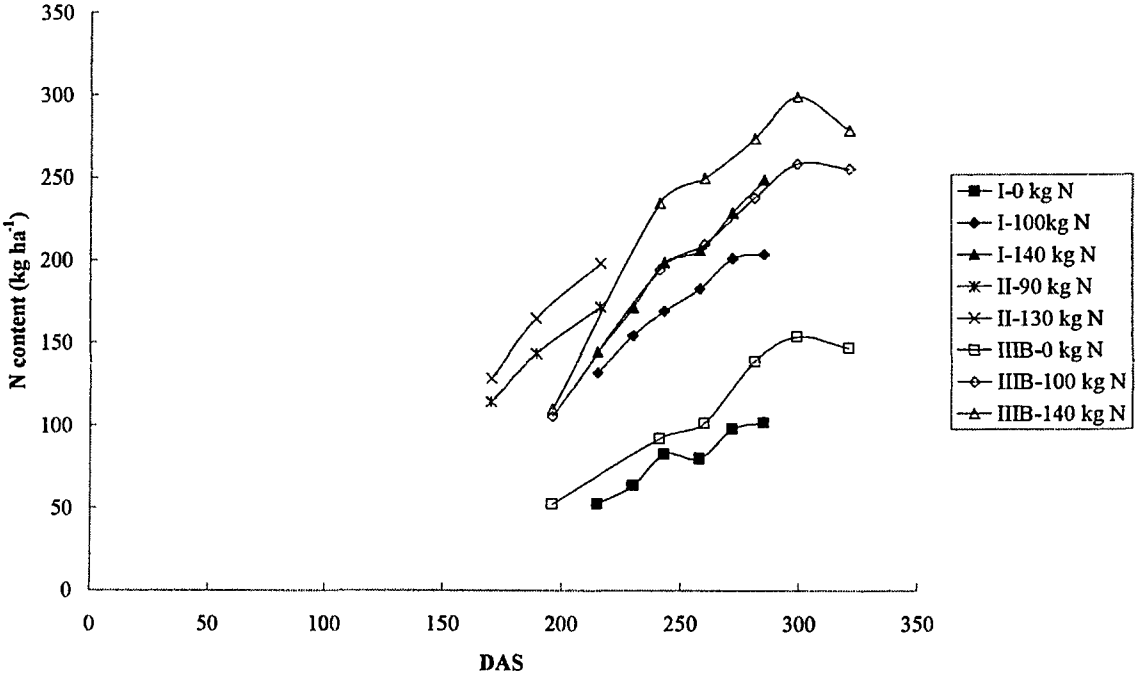


Figure 4.3.1.1 (b) The time change in aboveground N content

kg N: kg N ha^{-1}

4.3.1.2 Grain N content

There was no interaction in grain N content between fungicide programmes and N rates in Cycle I and Cycle II. In Cycle III-B, a statistically significant interaction was found between fungicide programmes and N rates with respect to grain N content. The interaction appeared to be caused by the greater response to applied N shown in the plots treated with fungicides. The plots treated with epoxiconazole alone and a mixture of epoxiconazole and trifloxystrobin showed a greater grain N content than untreated plots by 18 kg ha^{-1} and 29 kg ha^{-1} respectively with the N application rate of 140 kg ha^{-1} at harvest, while there was no difference in grain N content between fungicide programmes when no N was applied (P = 0.040) (Fig. 4.3.1.2 (a)). The plots treated with a mixture of epoxiconazole and trifloxystrobin showed a greater grain N content than untreated plots with the N rate of 100 kg ha^{-1} as well (P = 0.040) (Fig. 4.3.1.2 (a)). A significant interaction was observed between varieties and fungicide programmes in Cycle III-C. The interaction appeared to be caused by a greater response of grain N content of Equinox to a mixture of epoxiconazole and trifloxystrobin than that to epoxiconazole alone and to a mixture of epoxiconazole and kresoxim-methyl. The plots treated with a mixture of epoxiconazole and trifloxystrobin showed a greater grain N content than those treated with epoxiconazole alone and with a mixture of epoxiconazole and kresoxim-methyl for Equinox by 15 kg ha^{-1} and 14 kg ha^{-1} respectively but not for Hereward (P = 0.029) (Fig. 4.3.1.2 (b)).

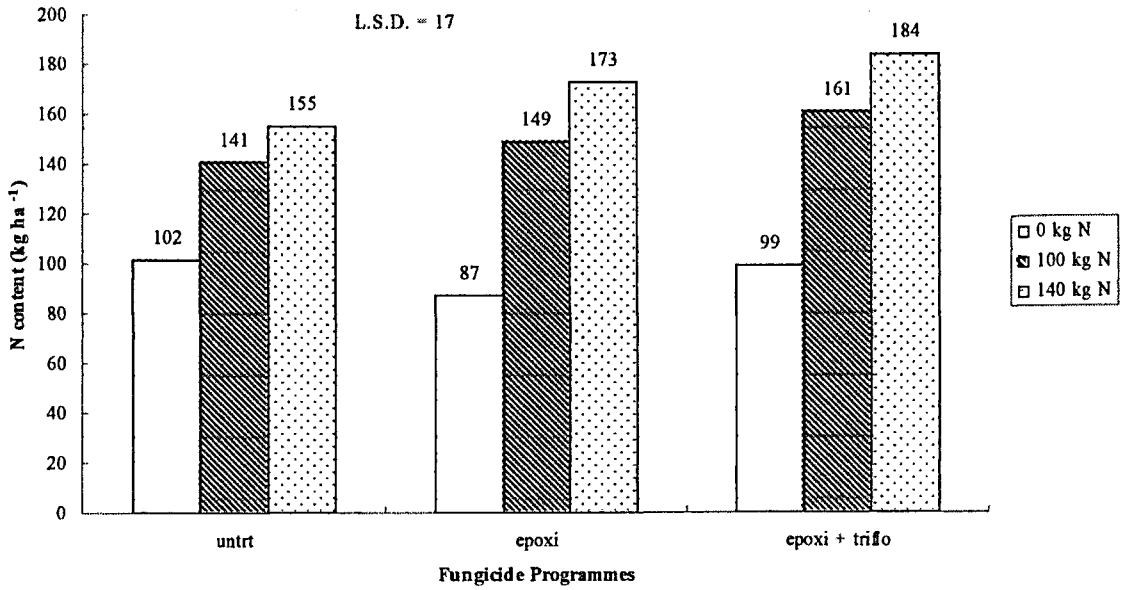


Figure 4.3.1.2 (a) The interactions in grain N content between fungicide programmes and N rates at combine-harvest in Cycle III-B

untrt: untreated; epoxi: epoxiconazole; triflo: trifloxystrobin

kg N: kg N ha^{-1}

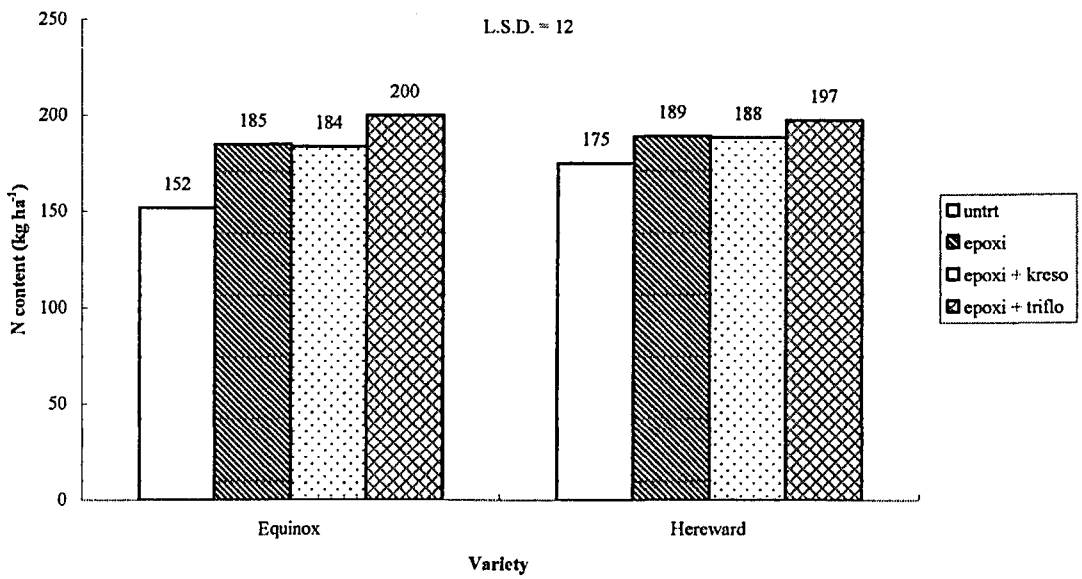


Figure 4.3.1.2 (b) The interaction in grain N content between varieties and fungicide programmes at combine-harvest in Cycle III-C

untrt: untreated; epoxi: epoxiconazole; kreso: kresoxim-methyl; triflo: trifloxystrobin

Fungicide Programmes

In Cycle I, the plots treated with a mixture of epoxiconazole and trifloxystrobin showed a greater N content of manually-harvested grains at pre-harvest than untreated plots and those treated with a mixture of epoxiconazole and kresoxim-methyl by 16 kg ha^{-1} and 12 kg ha^{-1} respectively ($P = 0.039$) (Table 4.3.1.2 (a)). Both in Cycle II and Cycle III-B, there was no difference in N content of manually-harvested grains at pre-harvest between fungicide programmes (Table 4.3.1.2 (a)). In Cycle III-C, untreated plots showed a much lower N content of manually-harvested grains at pre-harvest than those treated with fungicides with the difference ranging from 36 to 47 kg ha^{-1} ($P < 0.001$), while no difference was observed between fungicide treatments (Table 4.3.1.2 (a)).

Table 4.3.1.2 (a) Manually-harvested grain N content for fungicide programmes (kg ha^{-1})

Fungicide Programmes	*Cycle I*	*Cycle II*	*Cycle III-B*	*Cycle III-C*
untrt	123	147	136	162
epoxi	129	152	144	203
epoxi + kreso	127	149	-	198
epoxi + triflo	139	146	152	209
P value	= 0.039	= 0.711	= 0.093	< 0.001
L.S.D.	11	NS	NS	13
CV %	8.6	11.5	11.8	9.7

untrt: untreated; epoxi: epoxiconazole; kreso: kresoxim-methyl; triflo: trifloxystrobin

In Cycle I, the plots treated with a mixture of epoxiconazole and trifloxystrobin showed a greater N content of combine-harvested grains at harvest than untreated plots, those treated with epoxiconazole alone and those treated with a mixture of epoxiconazole and kresoxim-methyl by 6 kg ha^{-1}, 9 kg ha^{-1} and 9 kg ha^{-1}

respectively (P = 0.021) (Table 4.3.1.2 (b)). In Cycle III-B, the plots treated with a mixture of epoxiconazole and trifloxystrobin showed a greater N content of combine-harvested grains at harvest compared to untreated plots and those treated with epoxiconazole alone by 15 kg ha^{-1} and 12 kg ha^{-1} respectively (P = 0.009) (Table 4.3.1.2 (b)). In Cycle III-C, untreated plots showed a lower N content of combine-harvested grains at harvest than those treated with fungicide programmes with the difference ranging from 22 to 34 kg ha^{-1} (P < 0.001) (Table 4.3.1.2 (b)). Between fungicide programmes the plots treated with a mixture of epoxiconazole and trifloxystrobin showed a greater N uptake of combine-harvested grains at harvest than those treated with epoxiconazole alone and those with a mixture of epoxiconazole and kresoxim-methyl by 11 kg ha^{-1} and 12 kg ha^{-1} respectively (P < 0.001) (Table 4.3.1.2 (b)).

Table 4.3.1.2 (b) Combine-harvested grain N content for fungicide programmes (kg ha^{-1})

Fungicide Programmes	*Cycle I*	*Cycle III-B*	*Cycle III-C*
untrt	131	133	164
epoxi	128	136	187
epoxi + kreso	128	-	186
epoxi + triflo	137	148	198
P value	= 0.021	= 0.009	< 0.001
L.S.D.	6	10	8
CV %	4.5	8.4	6.3

untrt: untreated; epoxi: epoxiconazole; kreso: kresoxim-methyl; triflo: trifloxystrobin

N rates

The greater the N rate, the greater the N content of manually-harvested grains at pre-harvest for the four field experiments. The differences in N content of manually-harvested grains at pre-harvest between N rates

were all significant for each field experiment at the 0.1 % level. Similarly the greater the N rate, the greater the N content of combine-harvested grains at harvest for the four field experiments. The differences in N content of combine-harvested grains at harvest between N rates were all significant for each field experiment at the 0.1 % level.

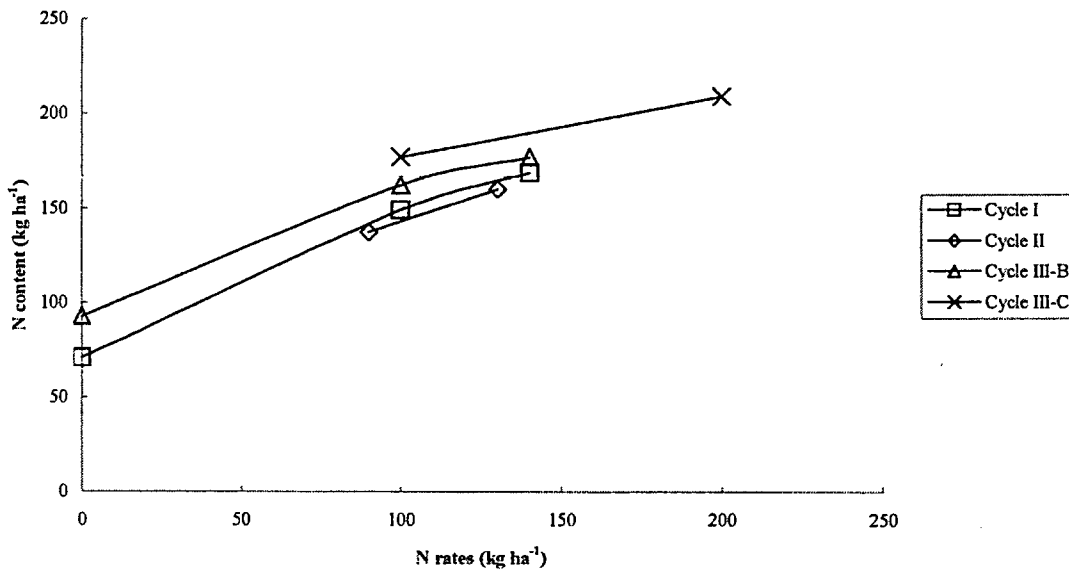


Figure 4.3.1.2 (c) The response of N content of manually-harvested grains to N rates at pre-harvest

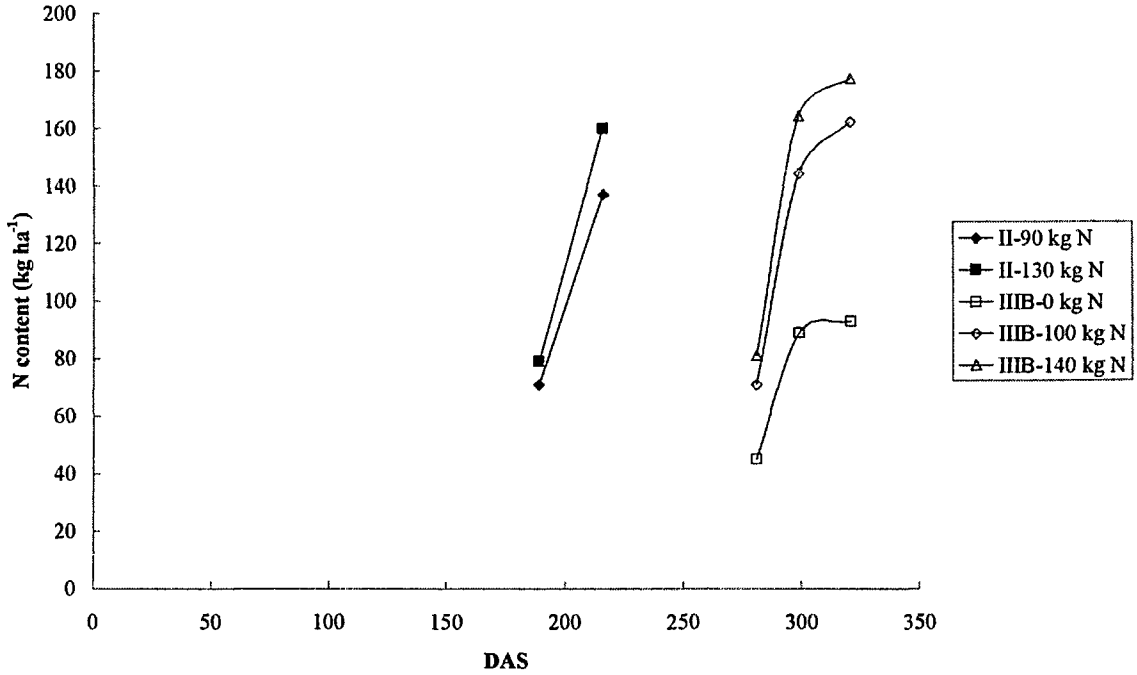


Figure 4.3.1.2 (d) The time change in grain N content in Cycle II and Cycle III-B

kg N: kg N ha^{-1}

4.3.2 Nitrogen Harvest Index (NHI) at Pre-harvest

In Cycle II a statistically significant interaction was observed in NHI between fungicide programmes and N rates at pre-harvest. The interaction was caused by a greater response of NHI to the applied N when treated with a mixture of epoxiconazole and trifloxystrobin compared to untreated plots. The plots treated with a mixture of epoxiconazole and trifloxystrobin showed a greater NHI than untreated plots by 0.010 with the N application rate of 130 kg ha^{-1} (P = 0.032) and for the plots treated with a mixture of epoxiconazole and trifloxystrobin, the plots treated with the N rate of 130 kg ha^{-1} showed a greater NHI than those treated with that of 90 kg ha^{-1} by 0.016 (P = 0.032) (Fig. 4.3.2 (a)). In Cycle III-C, there was a statistically significant interaction in NHI at pre-harvest between varieties and fungicide programmes. The interaction was caused by a greater response of NHI of Equinox to a mixture of epoxiconazole and trifloxystrobin than that to a

mixture of epoxiconazole and kresoxim-methyl. The plots treated with a mixture of epoxiconazole and trifloxystrobin showed a greater NHI than those treated with a mixture of epoxiconazole and kresoxim-methyl by 0.039 for Equinox but not for Hereward at pre-harvest ($P = 0.044$) (Fig. 4.3.2 (b)). No interactions were observed in NHI between fungicide programmes and N rates in Cycle III-B.

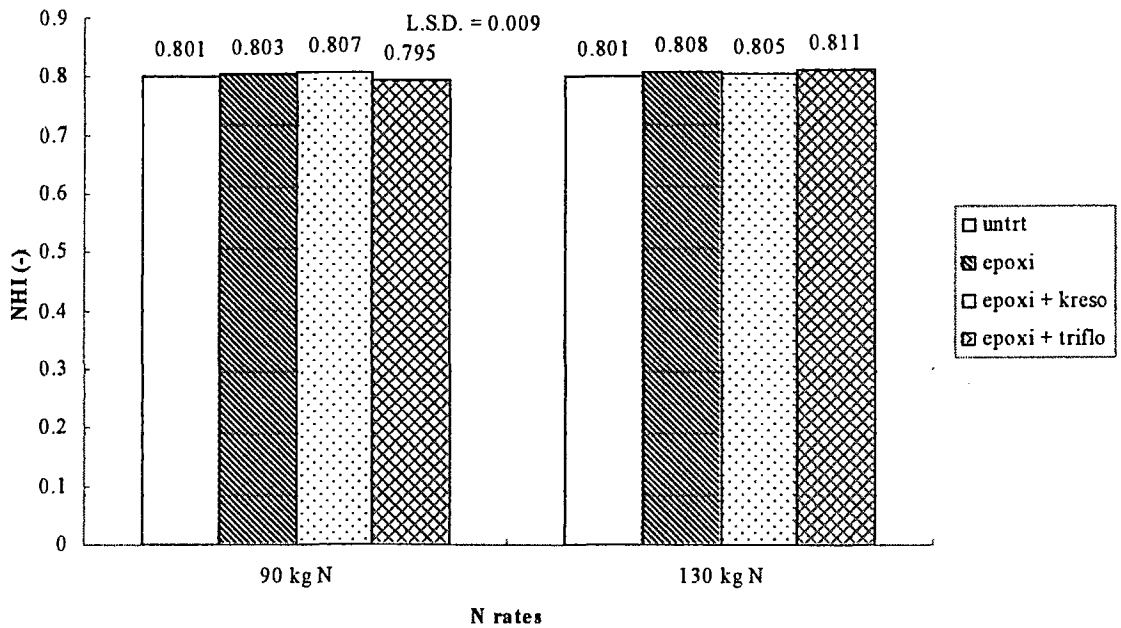


Figure 4.3.2 (a) The interaction in NHI between fungicide programmes and N rates at pre-harvest in Cycle II

untrt: untreated; epoxi: epoxiconazole; kreso: kresoxim-methyl; triflo: trifloxystrobin

kg N: kg N ha^{-1}

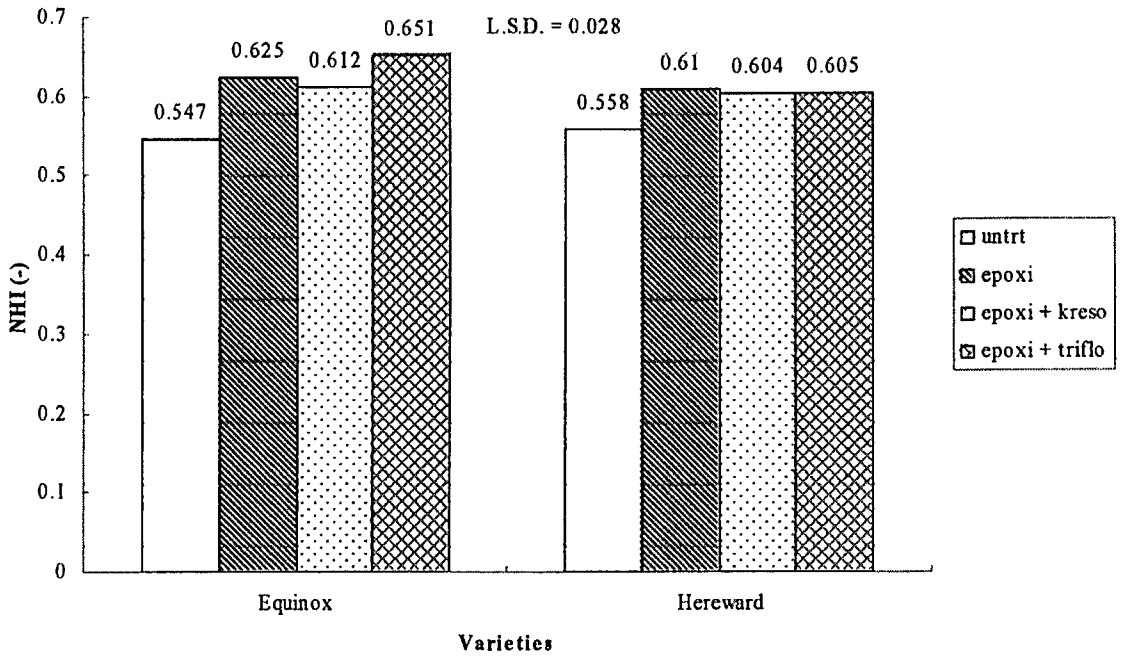


Figure 4.3.2 (b) The interaction in NHI between varieties and fungicide programmes at pre-harvest in Cycle III-C

untrt: untreated; epoxi: epoxiconazole; kreso: kresoxim-methyl; triflo: trifloxystrobin

Fungicide Programmes

In Cycle II, there was no difference in NHI between fungicide programmes (Table 4.3.2 (a)). In Cycle III-B, untreated plots showed a lower NHI than the plots treated with epoxiconazole alone and those treated with a mixture of epoxiconazole and trifloxystrobin by 0.024 and 0.043 respectively ($P < 0.001$) (Table 4.3.2 (a)). In Cycle III-C, untreated plots showed the lowest NHI compared to the plots treated with fungicides with the difference ranging from 0.056 to 0.076 ($P < 0.001$) (Table 4.3.2 (a)). The plots treated with a mixture of epoxiconazole and trifloxystrobin showed a greater NHI than those treated with a mixture of epoxiconazole and kresoxim-methyl by 0.020 ($P < 0.001$) (Table 4.3.2 (a)).

Table 4.3.2 (a) NHI of manually – harvested crops for fungicide programmes

Fungicide Programmes	*Cycle II*	*Cycle III-B*	*Cycle III-C*
untrt	0.801	0.612	0.552
epoxi	0.805	0.636	0.618
epoxi + kreso	0.806	-	0.608
epoxi + triflo	0.803	0.655	0.628
P value	= 0.398	< 0.001	< 0.001
L.S.D.	NS	0.020	0.020
CV %	1.2	3.8	4.6

untrt: untreated; epoxi: epoxiconazole; kreso: kresoxim-methyl; triflo: trifloxystrobin

N rates

There was no difference in NHI between N rates both in Cycle II and Cycle III-B (Table 4.3.2 (b)). In Cycle III-C, NHI was greater for the N rate of 100 kg ha^{-1} than for that of 200 kg ha^{-1} by 0.026 ($P < 0.001$) (Table 4.3.2 (b)).

Table 4.3.2 (b) NHI of manually – harvested crops for N rates

N rates (kg ha^{-1})	*Cycle II*	*Cycle III-B*	*Cycle III-C*
0	-	0.634	-
90	0.801	-	-
100	-	0.635	0.615
130	0.806	-	-
140	-	0.634	-
200	-	-	0.589
P value	= 0.051	= 0.996	< 0.001
L.S.D.	NS	NS	0.014
CV %	1.2	3.8	4.6

Time Change in NHI

The time change in NHI for fungicide programmes in Cycle III-B during grain filling period is shown in Figure 4.3.2 (c). The difference in NHI between fungicide programmes increased in the later part of grain filling and at pre-harvest untreated plots showed a smaller NHI than those treated with epoxiconazole alone and with a mixture of epoxiconazole and trifloxystrobin as it has been already mentioned in Table 4.3.2 (c).

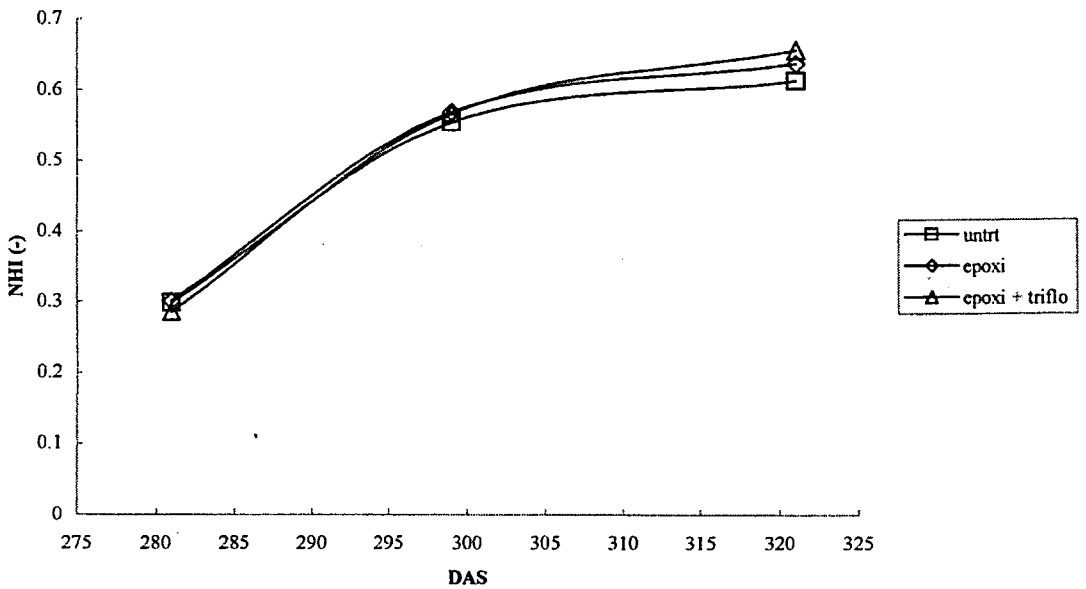


Figure 4.3.2 (c) The time change in NHI for fungicide programmes in Cycle III-B

untrt: untreated; epoxi: epoxiconazole; triflo: trifloxystrobin

4.3.3 N partitioning in Canopy

4.3.3.1 Stem N content

No interactions were observed in stem N content between fungicide programmes and N rates at any times of

sampling in Cycle I and Cycle III-B. In Cycle II, an interaction was observed in N content of lower stem between fungicide programmes and N rates at 168 – 172 days after sowing (i.e. at anthesis). The interaction was caused by a greater response of lower stem N content to a mixture of epoxiconazole and kresoxim-methyl than untreated and that to other fungicide programmes. The plots treated with a mixture of epoxiconazole and kresoxim-methyl showed a greater N content of lower stem than untreated plots, those treated with epoxiconazole alone and those treated with a mixture of epoxiconazole and trifloxystrobin with the difference ranging from 1.9 to 2.8 kg ha^{-1} when treated with the N application rate of 130 kg ha^{-1} (P = 0.009) (Fig. 4.3.3.1 (a)).

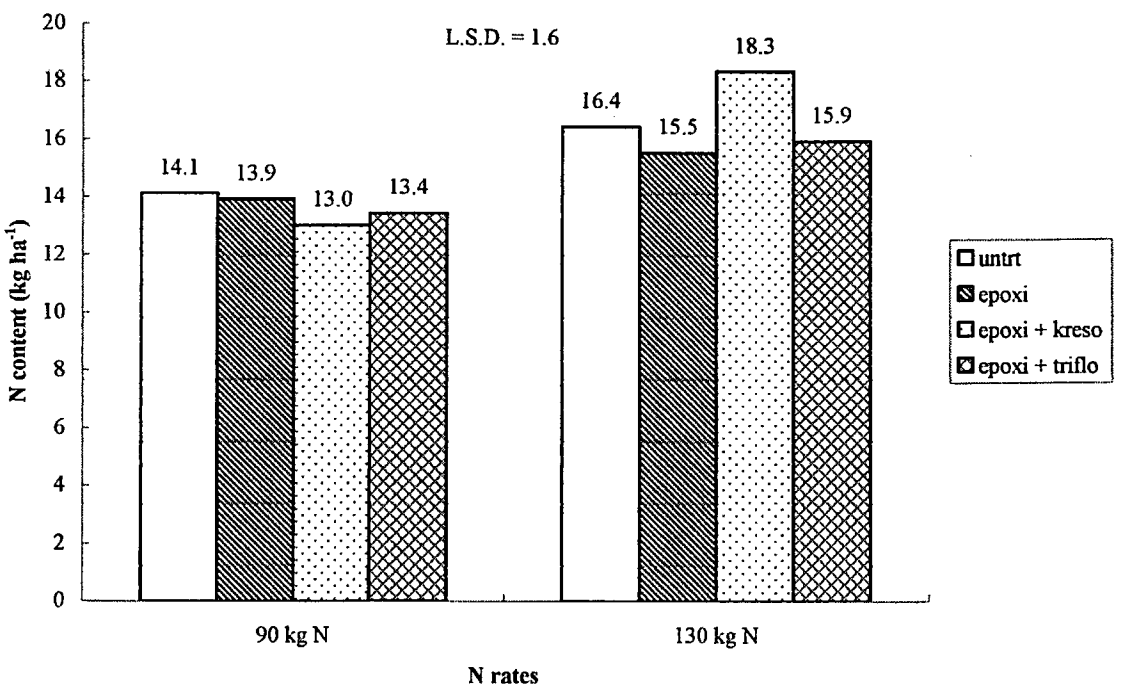


Figure 4.3.3.1 (a) The interaction in lower stem N content between fungicide programmes and N rates at 168 – 172 DAS (after anthesis) in Cycle II

untrt: untreated; epoxi: epoxiconazole; kreso: kresoxim-methyl; triflo: trifloxystrobin

kg N: kg N ha^{-1}

Fungicide Programmes

There was no difference in stem N content between fungicide programmes at any times of sampling in any field experiment (data not shown).

N rates

General trend for all the field experiments was that the greater the N rate, the greater the stem N content (data not shown).

Time Change in Stem N content

Stem N content was the highest around the time of anthesis and then declined towards harvest. In Cycle III-B stem N content was much greater than that in Cycle I even though the two field experiments received the same rates of N fertilizer.

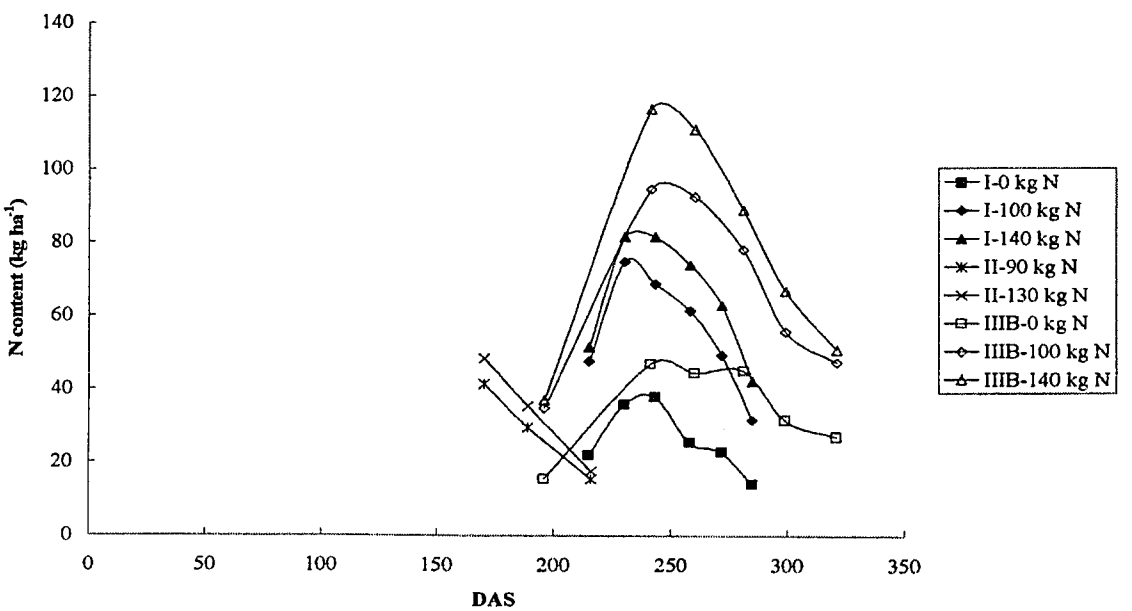


Figure 4.3.3.1 (b) The time change in stem N content in Cycle I, Cycle II and Cycle III-B

kg N: kg N ha^{-1}

4.3.3.2 Leaf N content

No interaction was observed in leaf N content between fungicide programmes and N rates at any times of sampling in Cycle I and Cycle III-B. In Cycle II, an interaction was observed in N content of lower leaf between fungicide programmes and N rates at 168 – 172 days after sowing (i.e. after anthesis). The interaction appeared to be caused by a greater response of N content of upper leaf to a mixture of epoxiconazole and kresoxim-methyl than untreated and that to epoxiconazole alone. With the N application rate of 130 kg ha^{-1}, the plots treated with a mixture of epoxiconazole and kresoxim-methyl showed a greater N content of upper leaf than untreated plots and those treated with epoxiconazole alone by 2.9 kg ha^{-1} and 1.9 kg ha^{-1} respectively and the plots treated with a mixture of epoxiconazole and trifloxystrobin showed a greater N content of upper leaf than untreated plots by 2.4 kg ha^{-1} ($P = 0.043$) (Fig. 4.3.3.2 (a)).

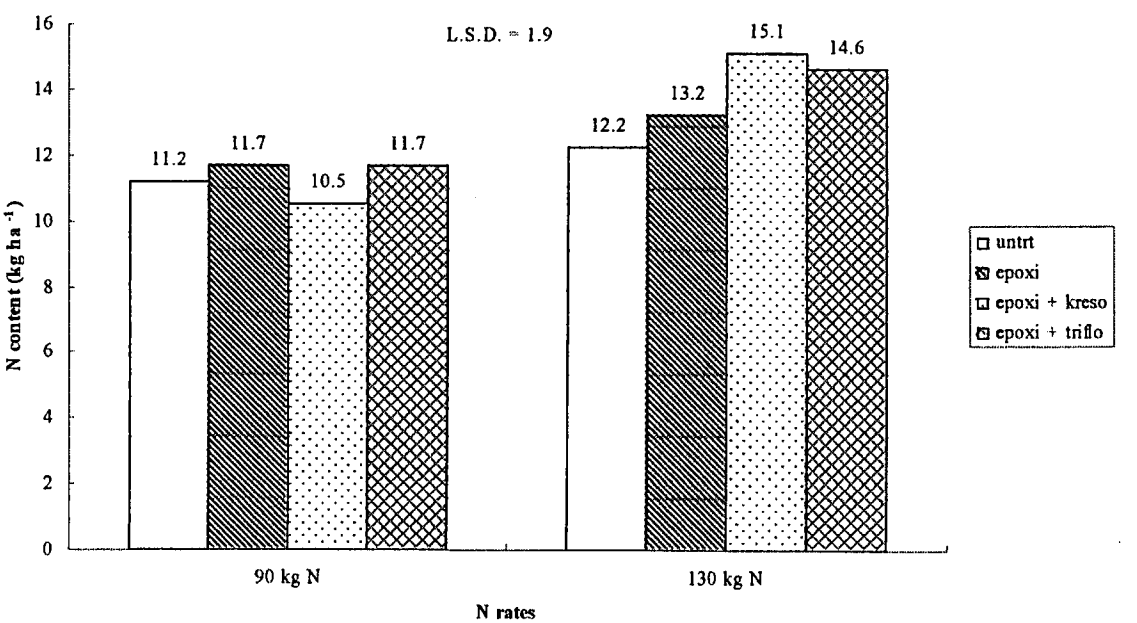


Figure 4.3.3.2 (a) The interaction in lower leaf N content between fungicide programmes and N rates at 168 – 172 DAS (after anthesis) in Cycle II

untrt: untreated; epoxi: epoxiconazole; kreso: kresoxim-methyl; triflo: trifloxystrobin

kg N: kg N ha^{-1}

Fungicide Programmes

In Cycle I at 229 – 232 days after sowing (i.e. at approximately two weeks before anthesis), the plots treated with a mixture of epoxiconazole and trifloxystrobin showed a greater N content of green leaf than untreated plots and those treated with a mixture of epoxiconazole and kresoxim-methyl by 6.1 kg ha^{-1} and 9.0 kg ha^{-1} respectively ($P < 0.001$) (Table 4.3.3.2 (a)). The plots treated with epoxiconazole alone showed a greater N content of green leaf than those treated with a mixture of epoxiconazole and kresoxim-methyl by 5.7 kg ha^{-1} ($P < 0.001$) (Table 4.3.3.2 (a)). N content in senesced leaf was lower for the plots treated with a mixture of epoxiconazole and kresoxim-methyl than for untreated plots and those treated with epoxiconazole alone by 1.1 kg ha^{-1} and 0.7 kg ha^{-1} respectively ($P = 0.014$) (Table 4.3.3.2 (a)). At 257 – 259 days after sowing (i.e. at approximately two weeks after anthesis), N content of senesced leaf was lower for the plots treated with a mixture of epoxiconazole and kresoxim-methyl and those treated with a mixture of epoxiconazole and trifloxystrobin than for untreated plots by 1.1 kg ha^{-1} ($P = 0.007$) and than those treated with epoxiconazole alone by 0.8 kg ha^{-1} ($P = 0.007$) (Table 4.3.3.2 (a)). At 271 – 273 days after sowing (i.e. at approximately four weeks after anthesis), N content of senesced leaf was lower for the plots treated with a mixture of epoxiconazole and kresoxim-methyl and those with a mixture of epoxiconazole and trifloxystrobin than for untreated plots by 2.4 kg ha^{-1} ($P = 0.001$) (Table 4.3.3.2 (a)). At the same time, the plots treated with a mixture of epoxiconazole and trifloxystrobin showed a greater N content in green leaf than untreated plots, those treated with epoxiconazole alone and those treated with a mixture of epoxiconazole and kresoxim-methyl by 5.1 kg ha^{-1}, 4.2 kg ha^{-1} and 4.7 kg ha^{-1} respectively ($P < 0.001$) (Table 4.3.3.2 (a)). At 284 – 286 days after sowing (i.e. at approximately six weeks after anthesis), N content of total leaf was

greater for untreated plots and those treated with a mixture of epoxiconazole and trifloxystrobin than for those treated with epoxiconazole alone by 2.3 kg ha^{-1} and by 2.1 kg ha^{-1} respectively ($P = 0.004$) and than for those treated with a mixture of epoxiconazole and kresoxim-methyl by 3.1 kg ha^{-1} and 2.9 kg ha^{-1} respectively ($P = 0.004$) (Table 4.3.3.2 (a)).

Table 4.3.3.2 (a) Leaf N content for fungicide programmes in Cycle I (kg ha^{-1})

Leaf	*DAS*	*untrt*	*epoxi*	*epoxi + kresoxim*	*epoxi + triflo*	P *value*	*L.S.D.*	*CV %*
*G Leaf**	*229-232*	56.7	59.5	53.8	62.8	< 0.001	3.8	6.7
	271-273	18.0	18.9	18.4	23.1	< 0.001	2.4	12.3
Sen	*229-232*	7.8	7.4	6.7	7.2	= 0.014	0.7	9.5
*Leaf***	*257-259*	9.2	8.9	8.1	8.1	= 0.007	0.7	8.4
	271-273	14.1	12.8	11.7	11.7	= 0.001	1.2	10.1
Total Leaf	*284-286*	18.7	16.4	15.6	18.5	= 0.004	1.8	10.7

untrt: untreated; epoxi: epoxiconazole; kreso: kresoxim-methyl; triflo: trifloxystrobin

*G Leaf: green leaf

**Sen Leaf: senesced leaf

In Cycle II no difference in leaf N content was found between fungicides at any sampling (data not shown). In Cycle III-B, no difference in N content between fungicide programmes was observed on leaf 1 and leaf 2 at any sampling (Table 4.3.3.2 (b)). At 255 – 264 days after sowing (i.e. at anthesis), untreated plots showed a lower N content of lower green leaf than the plots treated with epoxiconazole alone and those treated with a mixture of epoxiconazole and trifloxystrobin by 4.3 kg ha^{-1} and 3.2 kg ha^{-1} respectively ($P = 0.002$) (Table 4.3.3.2 (b)). At 269 – 288 days after sowing (i.e. at approximately three weeks after anthesis), N content of lower green leaf layer was lower for untreated plots than those treated with a mixture of

epoxiconazole and trifloxystrobin by 6.5 kg ha^{-1} ($P < 0.001$) (Table 4.3.3.2 (b)). At the same time, N content of lower senesced leaf was greater for untreated plots than those treated with epoxiconazole alone and those treated with a mixture of epoxiconazole and trifloxystrobin by 4.4 kg ha^{-1} and 2.6 kg ha^{-1} respectively ($P = 0.002$) (Table 4.3.3.2 (b)). At 311 – 323 days after sowing (i.e. at approximately eight weeks after anthesis), untreated plots showed a greater N content of senesced leaf than those treated with a mixture of epoxiconazole and trifloxystrobin by 2.7 kg ha^{-1} ($P = 0.038$) (Table 4.3.3.2 (b)).

Table 4.3.3.2 (b) Leaf N content for fungicide programmes in Cycle III-B (kg ha^{-1})

Leaf	*DAS*	*untrt*	*epoxi*	*epoxi + triflo*	P *value*	*L.S.D.*	*CV %*
*L G Leaf**	***255-264***	13.7	16.9	18.0	= 0.002	2.3	16.8
	269-288	4.2	6.2	10.7	< 0.001	2.3	38.1
*L Sen Leaf***	***269-288***	20.1	17.5	15.7	= 0.002	2.2	14.8
*Sen Leaf****	***311-323***	29.3	25.8	26.6	= 0.038	2.7	11.9

untrt: untreated; epoxi: epoxiconazole; kreso: kresoxim-methyl; triflo: trifloxystrobin

*L G Leaf: lower green leaf

**L Sen Leaf: lower senesced leaf

***Sen Leaf: senesced leaf

N rates

A similar trend to stem N content was observed in leaf N content that the greater the N rate, the greater the leaf N content. Maximum total leaf N content was achieved around the time of flag leaf emergence in Cycle I and Cycle III-B. This could not be confirmed with Cycle II where samples were taken only after anthesis (Fig. 4.3.3.2 (b)).

Time change in leaf N content with respect to LAI

Plotting leaf N content against LAI during the period from two weeks before anthesis to approximately three weeks after anthesis gives the three graphs of Figure 4.3.3.2 (c). The information as to significant difference in LAI and leaf N content can be found in Chapter 3 and in the present section of Chapter 4.

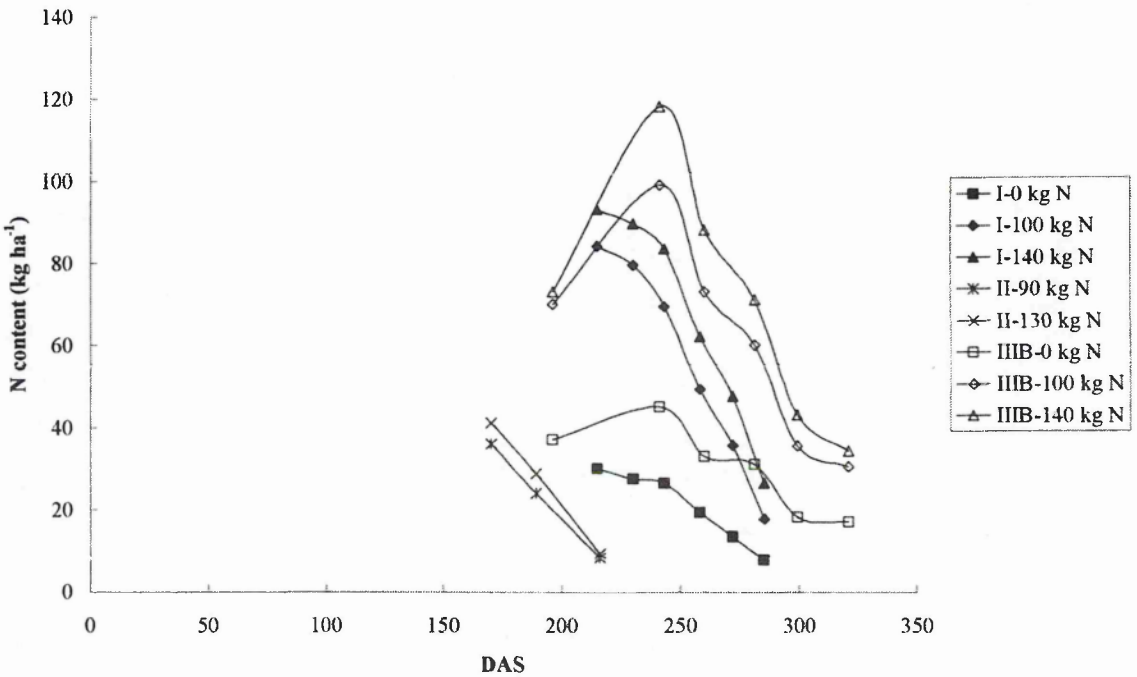


Figure 4.3.3.2 (b) The time change in total leaf N content in Cycle I, Cycle II and Cycle III-B

kg N: kg N ha^{-1}

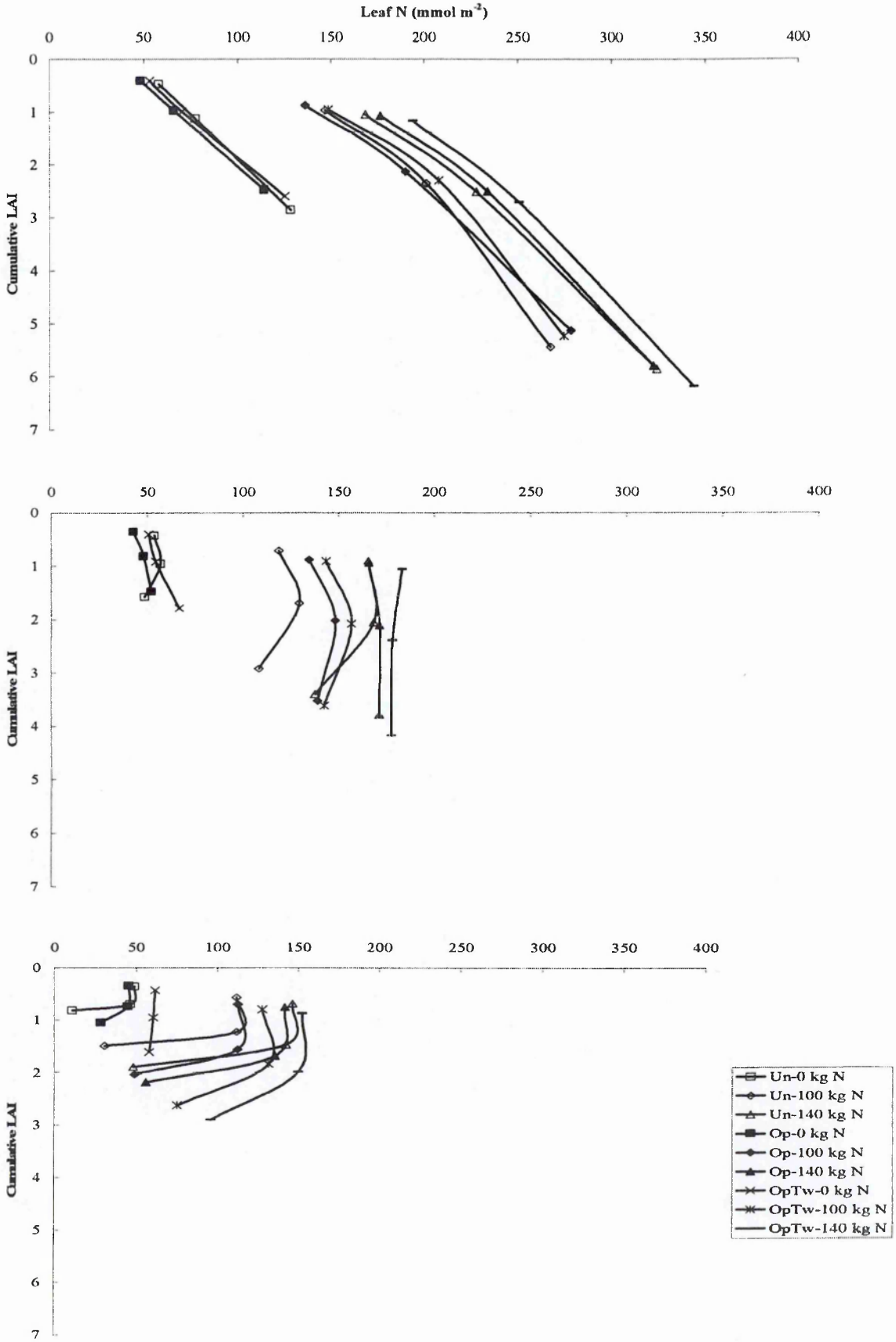


Figure 4.3.3.2 (c) Relationships between leaf N content and LAI of leaf layers in Cycle III-B

(top) 237 – 244 DAS (at approximately two weeks before anthesis)

(middle) 255 – 264 DAS (at anthesis),

(bottom) 269 – 288 DAS (at approximately three weeks after anthesis)

4.3.3.3 SLN of leaf layers in Cycle III-B

There was no interaction in SLN in any leaf layer between fungicide programmes and N rates at 237 – 244 days after sowing (i.e. at approximately two weeks before anthesis).

Fungicide Programmes

The application of a mixture of epoxiconazole and trifloxystrobin resulted in significantly greater SLN on leaf 1 than that of epoxiconazole alone by 6 mmol m^{-2} ($P = 0.05$) (Table 4.3.3.3 (a)). Similar differences were seen between the fungicide programmes on leaf 2 by 7.4 mmol m^{-2} ($P = 0.044$) and on green leaf by 5.0 mmol m^{-2} ($P = 0.03$) (Table 4.3.3.3 (a)).

Table 4.3.3.3 (a) Specific Leaf N (SLN) of the three leaf layers for fungicide programmes at 237 – 244 DAS (at approximately two weeks before anthesis) in Cycle III-B

Fungicide Programmes	*Leaf 1*	*Leaf 2*	*Lower Green Leaf*
untrt	144	140.4	86.0
epoxi	146	143.7	88.8
epoxi + triflo	150	147.8	91.0
P value	= 0.050	= 0.044	= 0.030
L.S.D.	5	5.7	3.6
CV %	4.1	4.7	4.8

untrt: untreated; epoxi: epoxiconazole; triflo: trifloxystrobin

N rates

In Cycle III-B at 237 – 244 days after sowing (i.e. at approximately two weeks before anthesis), the greater the N rate, the greater the SLN for all the three leaf layers ($P < 0.001$) (Table 4.3.3.3 (b)).

Table 4.3.3.3 (b) Specific Leaf N (SLN) of the three leaf layers for N rates at 237 – 244 DAS (at approximately two weeks before anthesis) in Cycle III-B

N rates ($kg\ ha^{-1}$)	*Leaf 1*	*Leaf 2*	*Lower Green Leaf*
0	122	117.8	76.4
100	154	151.5	91.4
140	164	162.4	98.0
P value	< 0.001	< 0.001	< 0.001
L.S.D.	5	5.7	3.6
CV %	4.1	4.7	4.8

4.3.4 Plant N concentration at Pre-Harvest and at Harvest

4.3.4.1 Grain N concentration at Pre-Harvest and at Harvest

Grain yields and grain N concentrations for manually-harvested grains at pre-harvest and combine-harvested grains at harvest were plotted against each other (Fig. 4.3.4.1 (a) – (e)). The range of grain N concentration suitable for bread-making wheat falls between 2.19 % and 2.59 % (@100 % DM), which is shown in the figures as two lines (Fig. 4.3.4.1 (a) – (e)). Comparing data sets of manually-harvested grains and those of combine-harvested grains for each field experiment, the variability was greater for manually-harvested data than combine-harvested data for all the field experiments (Fig. 4.3.4.1 (c), (d), (e)). This was confirmed by CV's when data sets were analyzed with ANOVA (Table 4.3.4.1 (a), (b)). In Cycle I, the CV of manually-harvested data was 3.2 %, while that of combine-harvested data was 2.4 %. In Cycle III-B, the former was 4.7 % and the latter was 2.5 %. In Cycle III-C, the former was 4.3 % and the latter was 3.3 %. In the case of manually-harvested grains, 5 plots out of 33 plots met the requirement of grain N concentration

for bread-making in Cycle I. It was 38 plots out of 72 plots in Cycle II, 3 plots out of 36 plots in Cycle III-B and 21 plots out of 61 plots in Cycle III-C. In the case of combine-harvested grains, no plots met the requirement in Cycle I. In Cycle III-B, only 1 plot met the requirement out of 36 plots. In Cycle III-C, 25 plots out of 64 plots met the requirement. The number of the plots that met the requirement for each treatment is found in Appendix 9.

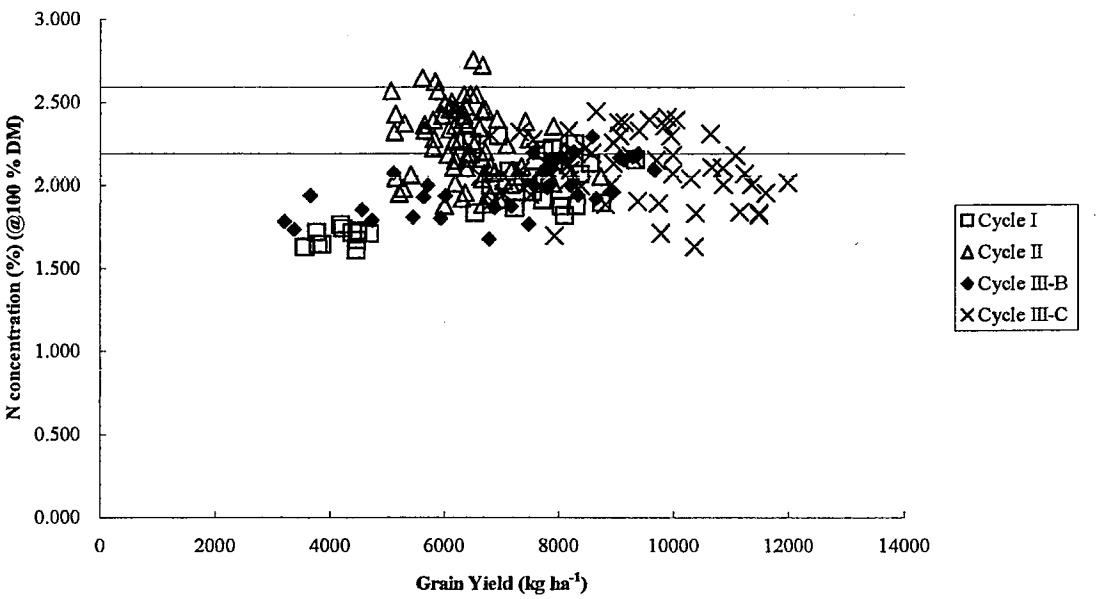


Figure 4.3.4.1 (a) The relationship between yield and N concentration of manually-harvested grains at pre-harvest

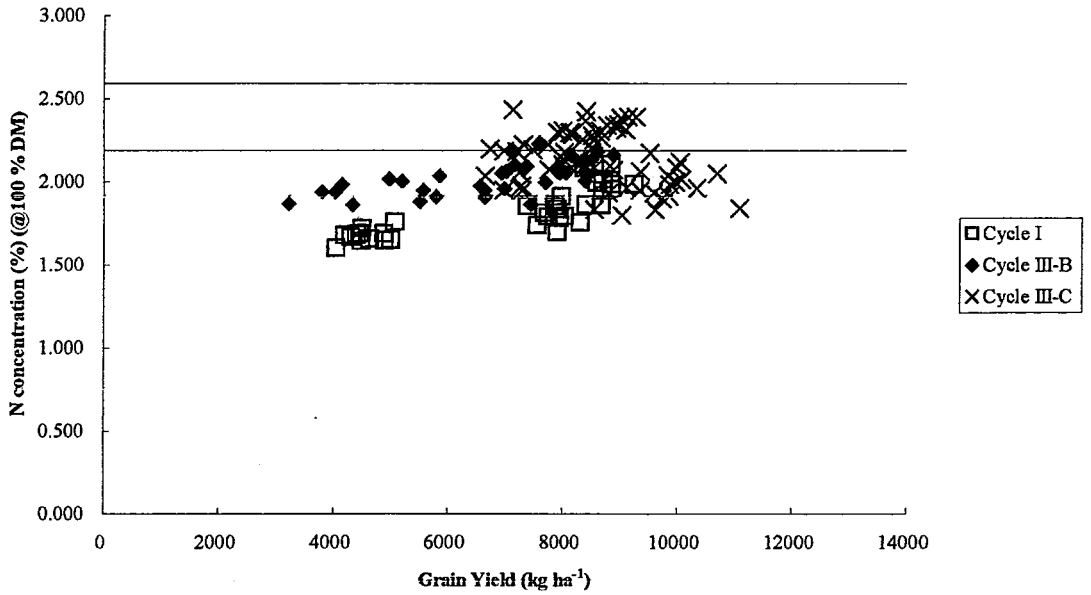


Figure 4.3.4.1 (b) The relationship between yield and N concentration of combine-harvested grains at harvest

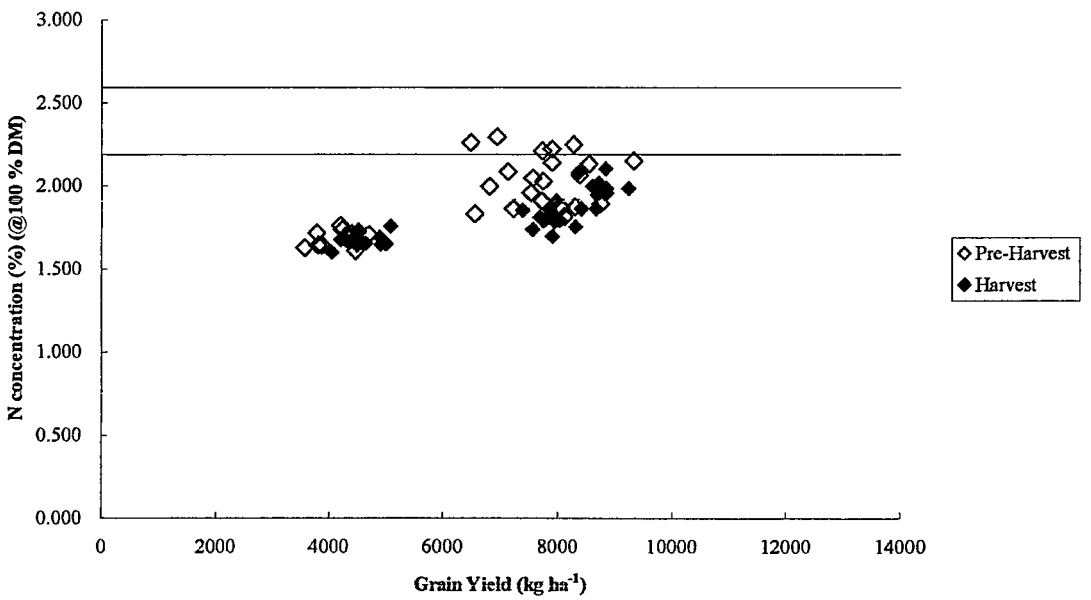


Figure 4.3.4.1 (c) The relationship between yield and N concentration of manually-harvested grains – Comparison between pre-harvest and harvest data sets in Cycle I

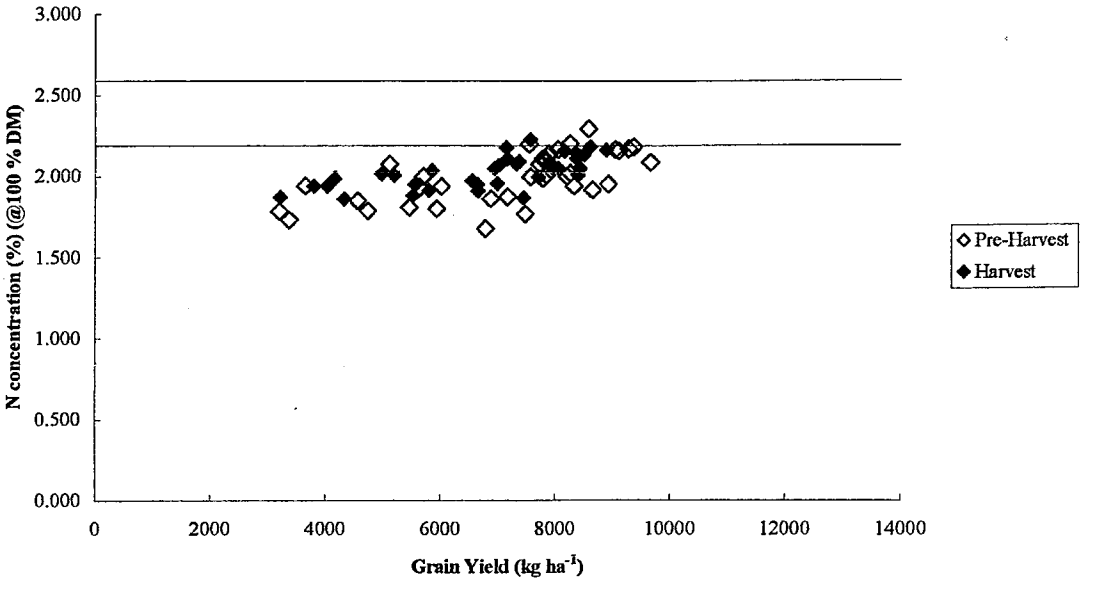


Figure 4.3.4.1 (d) The relationship between yield and N concentration of combine-harvested grains – Comparison between pre-harvest and harvest data sets in Cycle III-B

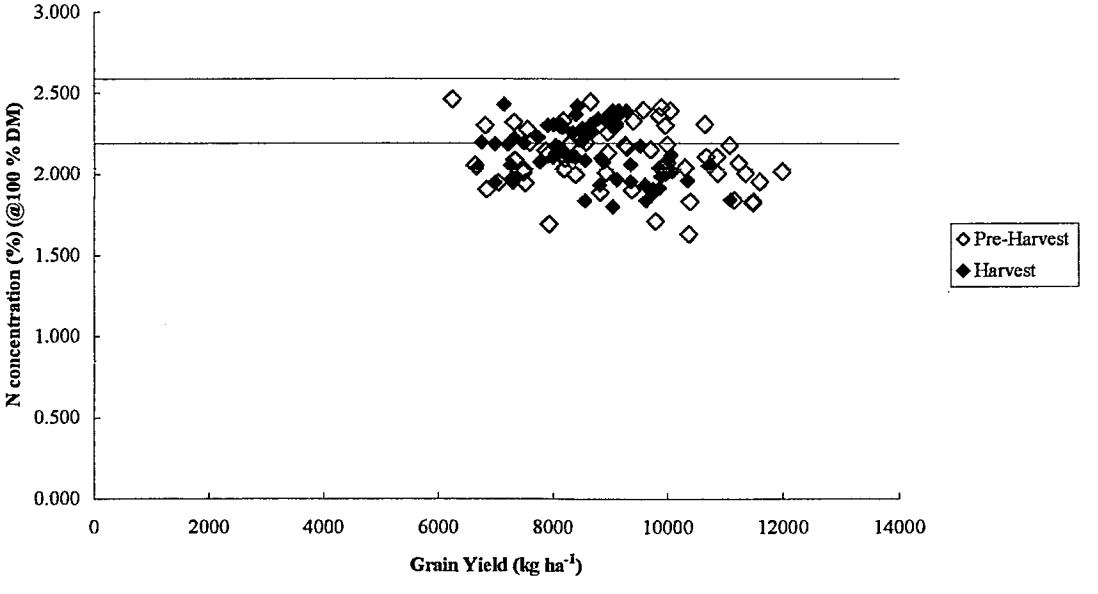


Figure 4.3.4.1 (e) The relationship between yield and N concentration of combine-harvested grains – Comparison between pre-harvest and harvest data sets in Cycle III-C

No interaction was observed in grain N concentration between fungicide programmes and N rates in Cycle I, Cycle II and Cycle III-B. In Cycle III-C, a statistically significant interaction was observed in N concentration of manually-harvested grains as well as combine-harvested grains between varieties and fungicide programmes. The interaction was caused by untreated plots of Equinox showing higher grain N concentration compared to fungicide programmes. Untreated plots showed a higher N concentration of manually-harvested grains than those treated with fungicide programmes for Equinox with the difference ranging from 0.10 % to 0.18 %, however, no difference in N concentration of manually-harvested grains between fungicide programmes was observed for Hereward ($P < 0.001$) (Fig. 4.3.4.1 (f)). For untreated plots, there was no difference in grain N concentration of manually-harvested grains between Hereward and Equinox ($P < 0.001$) (Fig. 4.3.4.1 (f)). Similarly to manually-harvested grains, there was no difference in N concentration of combine-harvested grains between fungicide programmes for Hereward, while for Equinox untreated plots showed a higher grain N concentration than the plots treated with fungicide programmes with the difference ranging from 0.11 % to 0.19 % ($P = 0.003$) and the plots treated with a mixture of epoxiconazole and kresoxim-methyl showed a lower grain N concentration than those treated with epoxiconazole alone by 0.08 % ($P = 0.003$) (Fig. 4.3.4.1 (g)).

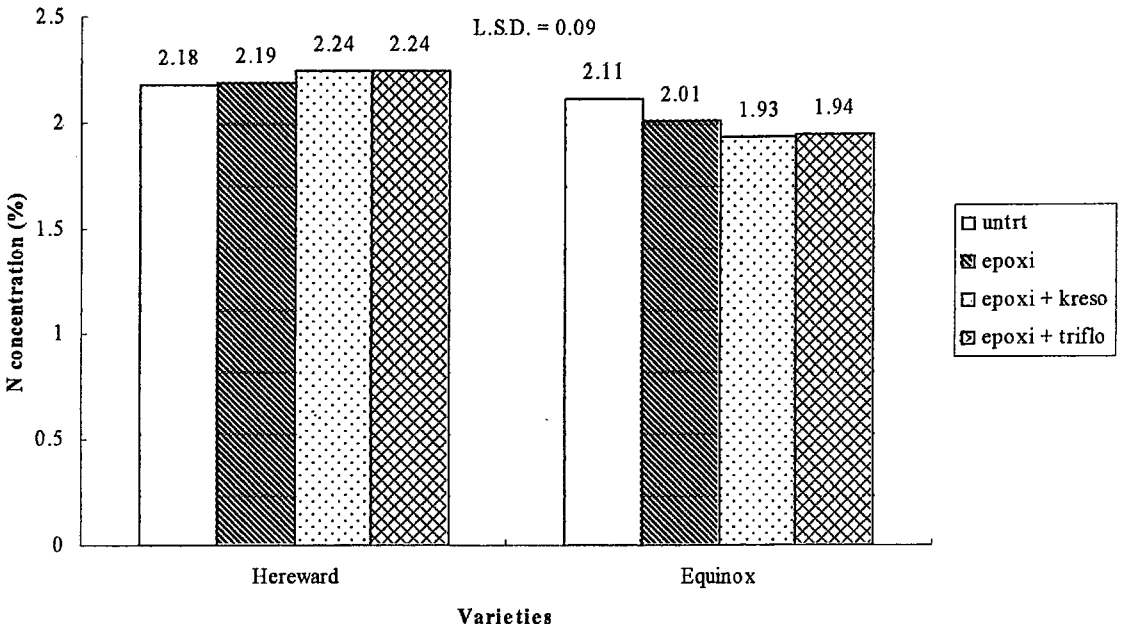


Figure 4.3.4.1 (f) The interaction in N concentration of manually-harvested grains between varieties and fungicide programmes in Cycle III-C

untrt: untreated; epoxi: epoxiconazole; kreso: kresoxim-methyl; triflo: trifloxystrobin

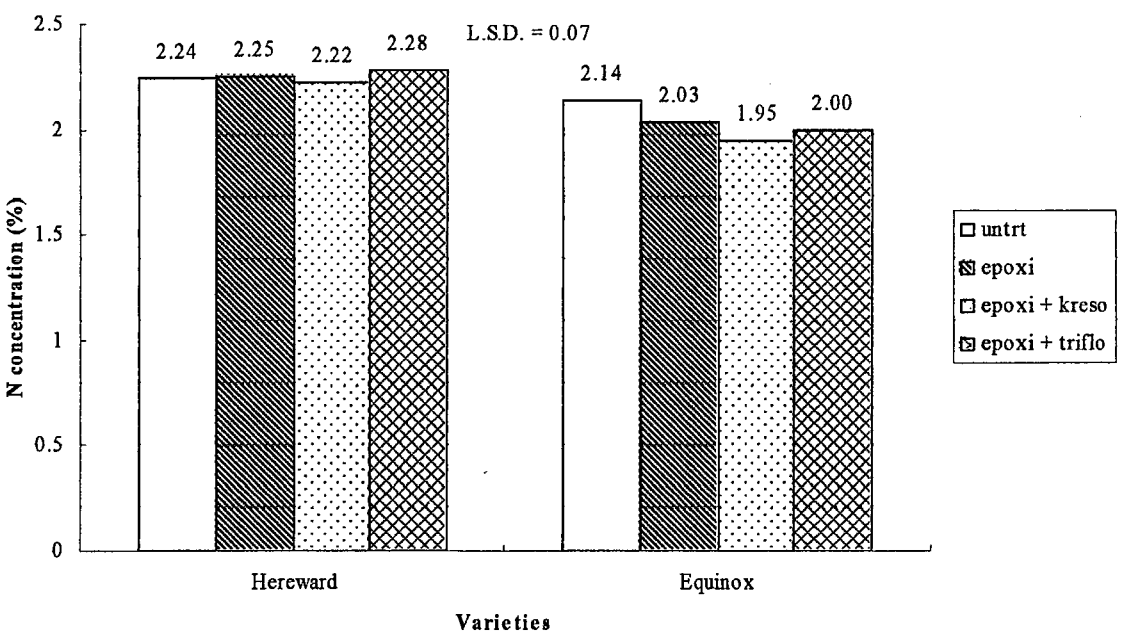


Figure 4.3.4.1 (g) The interaction in N concentration of combine-harvested grains between varieties and fungicide programmes in Cycle III-C

untrt: untreated; epoxi: epoxiconazole; kreso: kresoxim-methyl; triflo: trifloxystrobin

Fungicide Programmes

At pre-harvest, there was no difference in grain N concentration between fungicide programmes in any of the four field experiments (Table 4.3.4.1 (a)). In Cycle I, untreated plots showed a greater N concentration of combine-harvested grains compared to those treated with epoxiconazole alone and those with a mixture of epoxiconazole and kresoxim-methyl by 0.042 % and 0.067 % respectively (P = 0.013) (Table 4.3.4.1 (b)). The plots treated with a mixture of epoxiconazole and trifloxystrobin showed a greater grain N concentration than those treated with a mixture of epoxiconazole and kresoxim-methyl by 0.058 % (P = 0.013) (Table 4.3.4.1 (b)). No difference in combine-harvested grain N concentration was observed between fungicide programmes in Cycle III-B (Table 4.3.4.1 (b)). In Cycle III-C, untreated plots showed a greater N concentration of combine-harvested grains than those treated with fungicide programmes with the difference ranging from 0.05 % to 0.10 % (P = 0.004) (Table 4.3.4.1 (b)). The plots treated with a mixture of epoxiconazole and kresoxim-methyl showed a lower grain N concentration than those treated with epoxiconazole alone and those with a mixture of epoxiconazole and trifloxystrobin by 0.05 % and 0.05 % respectively (P = 0.004) (Table 4.3.4.1 (b)).

Table 4.3.4.1 (a) Manually-harvested grain N concentration for fungicide programmes (%)

Fungicide Programmes	*Cycle I*	*Cycle II*	*Cycle III-B*	*Cycle III-C*
untrt	1.93	2.23	1.94	2.15
epoxi	1.92	2.28	2.01	2.10
epoxi + kreso	1.93	2.29	-	2.08
epoxi + triflo	1.96	2.21	2.02	2.09
P value	= 0.632	= 0.100	= 0.119	= 0.245
L.S.D.	NS	NS	NS	NS
CV %	3.2	4.8	4.7	4.3

untrt: untreated; epoxi: epoxiconazole; kreso: kresoxim-methyl; triflo: trifloxystrobin

Table 4.3.4.1 (b) Combine-harvested grain N concentration for fungicide programmes (%)

Fungicide Programmes	*Cycle I*	*Cycle III-B*	*Cycle III-C*
untrt	1.856	2.047	2.19
epoxi	1.814	2.014	2.14
epoxi + kreso	1.789	-	2.09
epoxi + triflo	1.847	2.023	2.14
P value	= 0.013	= 0.259	= 0.004
L.S.D.	0.042	NS	0.05
CV %	2.4	2.5	3.3

untrt: untreated; epoxi: epoxiconazole; kreso: kresoxim-methyl; triflo: trifloxystrobin

N rates

The greater the N rate, the greater the N concentration of both manually-harvested grains and combine-harvested grains in any of the field experiments at the 0.1 % level of significance (Table 4.3.4.1 (c), Table 4.3.4.1 (d)).

Table 4.3.4.1 (c) Manually-harvested grain N concentration for N rates (%)

N rates ($kg\ ha^{-1}$)	*Cycle I*	*Cycle II*	*Cycle III-B*	*Cycle III-C*
0	1.68	-	1.86	-
90	-	2.16	-	-
100	1.93	-	1.97	1.96
130	-	2.35	-	-
140	2.19	-	2.15	-
200	-	-	-	2.25
P value	< 0.001	< 0.001	< 0.001	< 0.001
L.S.D.	0.05	0.05	0.08	0.05
CV %	3.2	4.8	4.7	4.3

Table 4.3.4.1 (d) Combine-harvested grain N concentration for N rates (%)

N rates ($kg\ ha^{-1}$)	*Cycle I*	*Cycle III-B*	*Cycle III-C*
0	1.671	1.945	-
90	-	-	-
100	1.805	2.007	2.06
130	-	-	-
140	2.002	2.132	-
200	-	-	2.22
P value	< 0.001	< 0.001	< 0.001
L.S.D.	0.037	0.043	0.04
CV %	2.4	2.5	3.3

4.3.4.2 N concentration of Vegetative Parts at Pre-Harvest

N concentration of vegetative parts (stem + leaf + chaff + rachis) at pre-harvest tended to be lower for Cycle II and to be greater for Cycle III-C. There was no interaction in N concentration of vegetative parts at pre-harvest between fungicide programmes and N rates in Cycle II, Cycle III-B and Cycle III-C.

Fungicide Programmes

In Cycle II, there was no difference in N concentration of vegetative parts at pre-harvest between fungicide programmes (Table 4.3.4.2 (a)). Both in Cycle III-B and Cycle III-C, untreated plots showed a greater N concentration of vegetative parts at pre-harvest than those treated with fungicide programmes with the difference ranging from 0.051 % to 0.093 % and from 0.09 % to 0.18 % respectively ($P = 0.001$ and $P < 0.001$, respectively) (Table 4.3.4.2 (a)). In Cycle III-C, the plots treated with a mixture of epoxiconazole and kresoxim-methyl showed a greater N concentration of vegetative parts at pre-harvest than those treated with a mixture of epoxiconazole and trifloxystrobin by 0.09 % ($P < 0.001$) (Table 4.3.4.2 (a)).

Table 4.3.4.2 (a) N concentration of vegetative parts (stem + leaf + chaff) at pre-harvest for fungicide programmes (%)

Fungicide Programmes	*Cycle II*	*Cycle III-B*	*Cycle III-C*
untrt	0.753	1.007	1.33
epoxi	0.739	0.956	1.21
epoxi + kreso	0.738	-	1.24
epoxi + triflo	0.747	0.914	1.15
P value	= 0.399	= 0.001	< 0.001
L.S.D.	NS	0.045	0.07
CV %	3.9	5.6	8.3

untrt: untreated; epoxi: epoxiconazole; kreso: kresoxim-methyl; triflo: trifloxystrobin

N rates

The greater the N rate, the greater the N concentration of vegetative parts at pre-harvest in any of the field experiments at the 0.1 % level of significance (Table 4.3.4.2 (b)).

Table 4.3.4.2 (b) N concentration of vegetative parts (stem + leaf + chaff) at pre-harvest for N rates (%)

N rates (kg ha^{-1})	*Cycle II*	*Cycle III-B*	*Cycle III-C*
0	-	0.778	-
90	0.716	-	-
100	-	0.984	1.07
130	0.773	-	-
140	-	1.116	-
200	-	-	1.39
P value	< 0.001	< 0.001	< 0.001
L.S.D.	0.014	0.045	0.05
CV %	3.9	5.6	8.3

4.3.4.3 N concentration of Aboveground Plant / N Use Efficiency (NUE) at Pre-Harvest

In Cycle II a statistically significant interaction was observed in N concentration of aboveground plant at pre-harvest between fungicide programmes and N rates. With the N application rate of 90 kg ha^{-1}, the plots treated with a mixture of epoxiconazole and kresoxim-methyl showed a greater aboveground N concentration (i.e. a smaller NUE) than those treated with epoxiconazole alone and those treated with a mixture of epoxiconazole and trifloxystrobin by 0.06 % (2.8 kg kg^{-1}) and 0.09 % (3.7 kg kg^{-1}) respectively (P = 0.030) (Fig. 4.3.4.3). There was no interaction in N concentration of aboveground plant at pre-harvest between fungicide programmes and N rates in Cycle III-B and Cycle III-C.

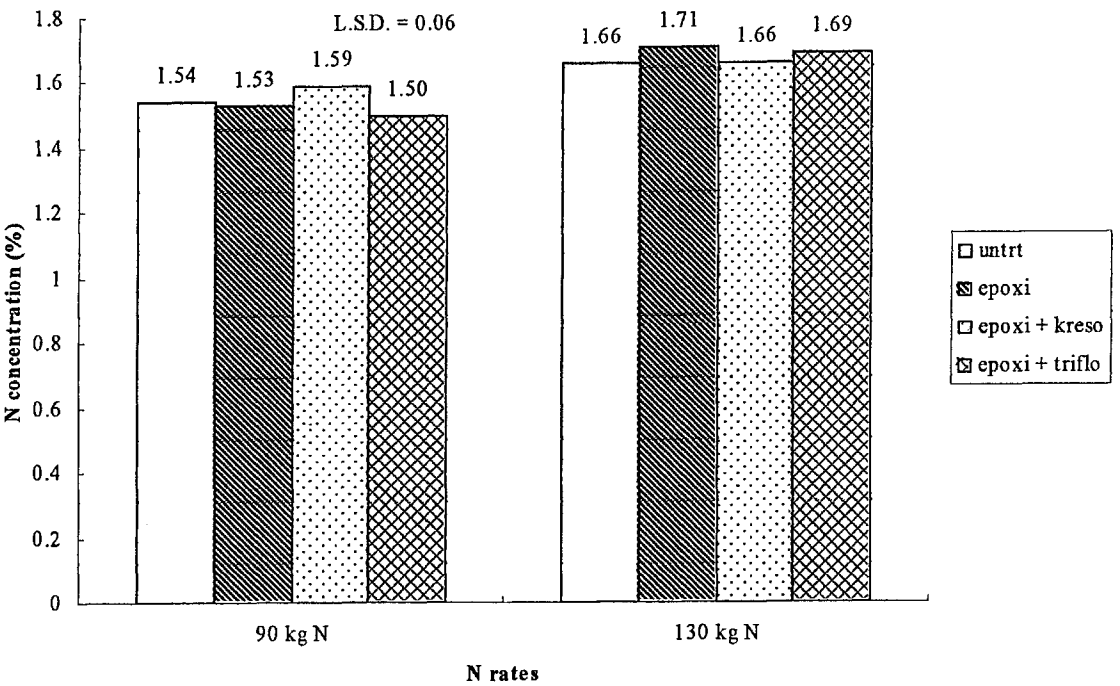


Figure 4.3.4.3 The interaction in aboveground N concentration between fungicide programmes and N rates at pre-harvest in Cycle II

untrt: untreated; epoxi: epoxiconazole; kreso: kresoxim-methyl; triflo: trifloxystrobin

kg N: kg N ha^{-1}

Fungicide Programmes

Both in Cycle II and Cycle III-B, there was no difference in N concentration of aboveground plant at pre-harvest between fungicide programmes (Table 4.3.4.3 (a)). In Cycle III-C, untreated plots showed a greater N concentration of aboveground plant at pre-harvest than those treated with a mixture of epoxiconazole and trifloxystrobin by 0.09 % ($P = 0.048$) (Table 4.3.4.3 (a)).

Table 4.3.4.3 (a) N concentration of aboveground plant at pre-harvest for fungicide programmes (%) (NUE (kg kg^{-1}))

Fungicide Programmes	*Cycle I**	*Cycle II*	*Cycle III-B*	*Cycle III-C*
untrt	1.031	1.60	1.43	1.69
	(99.3)	(62.7)	(71.2)	(60.1)
epoxi	0.978	1.62	1.43	1.64
	(105.6)	(62.3)	(70.9)	(61.6)
epoxi + kreso	0.956	1.63	-	1.64
	(107.9)	(61.8)		(62.0)
epoxi + triflo	0.966	1.60	1.42	1.60
	(106.3)	(63.1)	(71.5)	(63.1)
P value	= 0.003	= 0.504	= 0.888	= 0.048
	(= 0.008)	(= 0.507)	(= 0.930)	(= 0.097)
L.S.D.	0.038	NS	NS	0.06
	(4.9)			(NS)
CV %	3.9	4.2	4.6	4.9
	(4.7)	(4.3)	(5.2)	5.3

untrt: untreated; epoxi: epoxiconazole; kreso: kresoxim-methyl; triflo: trifloxystrobin

*data from 6th sampling

N rates

The higher the N rate, the greater the N concentration of aboveground plant at pre-harvest in any of the field experiments at the 0.1 % level of significance (Table 4.3.4.3 (b)).

Table 4.3.4.3 (b) N concentration of aboveground plant at pre-harvest for N rates (%) (NUE (kg kg^{-1}))

N rates (kg ha^{-1})	*Cycle I**	*Cycle II*	*Cycle III-B*	*Cycle III-C*
0	0.779	-	1.23	-
	(128.8)		(81.6)	
90	-	1.54	-	-
		(65.1)		
100	0.994	-	1.44	1.49
	(100.9)		(69.9)	(67.5)
130		1.68	-	-
		(59.7)		
140	1.174	-	1.61	-
	(84.6)		(62.4)	
200		-	-	1.79
				(55.9)
P value	< 0.001	< 0.001	< 0.001	< 0.001
L.S.D.	0.033	0.06	0.06	0.04
	(4.9)	(1.3)	(3.1)	(1.7)
CV %	3.9	4.2	4.6	4.9
	(4.7)	(4.3)	(5.2)	(5.3)

*data from 6th sampling

4.3.5 N Accumulation in relation to DM Accumulation

4.3.5.1 Pearson's Correlation Analysis between traits related to N and DM accumulations

The correlations between the traits related to DM and N accumulation as well as N concentration were analyzed according to McKendry *et al.* (1995) (Table 4.3.5.1 (a), (b), (c)). Grain yield (GY) and grain N concentration (GNC) were not correlated in Cycle II, while they were positively correlated in Cycle III-B ($P < 0.001$). Grain yield (GY) and grain N yield (GNY) were highly and positively correlated both in Cycle II

($P < 0.001$) and Cycle III-B ($P < 0.001$). More than 98 % of the variability in grain N yield (GNY) in this study was accounted for by total N at maturity (TNM) both in Cycle II and Cycle III-B. There was a moderate positive correlation between grain N concentration (GNC) and NHI in Cycle II, but these traits were not correlated in Cycle III-B.

Table 4.3.5.1 (a) Pearson's Correlation Analysis between traits related to DM and N accumulations in Cycle II (n = 72)

Trait	GNC	GNY	HI	NHI	SUM	TNM	TDMM	PAN	PADM	VNM
GY	NS	0.736***	0.306**	NS	NS	0.760***	0.937***	0.600***	0.836***	0.772***
GNC		0.522***	-0.551***	0.484***	NS	0.484***	NS	0.415***	NS	0.273*
GNY			NS	0.263*	NS	0.995***	0.812***	0.816***	0.718***	0.858***
HI				NS	0.915***	NS	NS	NS	NS	NS
NHI					0.555***	NS	NS	0.287*	NS	-0.265*
SUM						NS	NS	NS	NS	-0.293*
TNM							0.847***	0.801***	0.733***	0.906***
TDMM								0.641***	0.839***	0.890***
PAN									0.846***	0.648***
PADM										0.709***

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

GY = grain yield; GNC = grain N concentration; GNY = grain N yield (content); HI = Harvest Index; NHI = N Harvest Index; SUM = sum of HI and NHI; TNM = total plant N at maturity; TDMM = total dray matter at maturity; PAN = post-anthesis N accumulated; PADM = post-anthesis dry matter accumulated; VNM = vegetative N at maturity

Table 4.3.5.1 (b) Pearson's Correlation Analysis between traits related to DM and N accumulations for Hereward in Cycle II (n = 24)

Trait	GNC	GNY	HI	NHI	SUM	TNM	TDMM	PAN	PADM	VNM
GY	NS	0.847***	NS	-0.412*	-0.429*	0.870***	0.985***	0.658***	0.840***	0.906***
GNC		0.450*	NS	0.500*	NS	0.405*	NS	NS	NS	NS
GNY			NS	NS	NS	0.997***	0.828***	0.733***	0.655***	0.931***
HI				0.527**	0.913***	NS	-0.504*	NS	NS	-0.418*
NHI					0.828***	NS	-0.472*	NS	NS	-0.461*
SUM						NS	-0.559**	NS	-0.458*	-0.497*
TNM							0.855***	0.734***	0.679***	0.957***
TDMM								0.669***	0.850***	0.910***
PAN									0.829***	0.695***
PADM										0.730***

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

GY = grain yield; GNC = grain N concentration; GNY = grain N yield (content); HI = Harvest Index; NHI = N Harvest Index; SUM = sum of HI and NHI; TNM = total plant N at maturity; TDMM = total dray matter at maturity; PAN = post-anthesis N accumulated; PADM = post-anthesis dry matter accumulated; VNM = vegetative N at maturity

Table 4.3.5.1 (c) Pearson's Correlation Analysis between traits related to DM and N accumulations in Cycle III-B (n = 36)

Trait	GNC	GNY	HI	NHI	SUM	TNM	TDMM	PAN	PADM	VNM
GY	0.641***	0.976***	0.885***	NS	0.630***	0.967***	0.990***	NS	0.617***	0.897***
GNC		0.788***	0.682***	NS	0.545***	0.769***	0.592***	NS	NS	0.693***
GNY			0.883***	NS	0.641***	0.987***	0.955***	NS	0.531***	0.910***
HI				0.447***	0.859***	0.834***	0.818***	NS	0.453***	0.704***
NHI					0.842***	NS	NS	NS	NS	NS
SUM						0.525***	0.536***	NS	0.394*	NS
TNM							0.961***	NS	0.510***	0.964***
TDMM								NS	0.652***	0.915***
PAN									0.715***	NS
PADM										0.446**

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

GY = grain yield; GNC = grain N concentration; GNY = grain N yield (content); HI = Harvest Index; NHI = N Harvest Index; SUM = sum of HI and NHI; TNM = total plant N at maturity; TDMM = total dray matter at maturity; PAN = post-anthesis N accumulated; PADM = post-anthesis dry matter accumulated; VNM = vegetative N at maturity

4.3.5.2 Ratio of N Harvest Index (NHI) to Harvest Index (HI) at Pre-Harvest

In Cycle III-C, an interaction between varieties and fungicide programmes was observed. The interaction was caused by different response between Hereward and Equinox to fungicide programmes. Hereward showed a greater ratio of NHI to HI than Equinox for the plots treated with fungicides with the difference ranging from 0.072 to 0.111, but there was no difference in the ratio of NHI to HI between the two varieties for untreated plots ($P < 0.001$) (Fig. 4.3.5.2 (a)). For Hereward, untreated plots showed a smaller ratio of NHI to HI than those treated with fungicides with the difference ranging from 0.045 to 0.071, while for Equinox untreated plots showed a greater ratio than those treated with a mixture of epoxiconazole and kresoxim-methyl by 0.047 and there was no difference in the ratio between untreated plots and the plots treated with epoxiconazole alone and those treated with a mixture of epoxiconazole and trifloxystrobin ($P < 0.001$) (Fig. 4.3.5.2 (a)). For Equinox, the plots treated with a mixture of epoxiconazole and kresoxim-methyl showed a smaller ratio of NHI to HI than those treated with epoxiconazole alone and those treated with a mixture of epoxiconazole and trifloxystrobin by 0.032 and 0.039 respectively, however, for Hereward, there was no difference in the ratio of NHI to HI between the three fungicide treatments ($P < 0.001$) (Fig. 4.3.5.2 (a)). There were no interactions in ratio of NHI to HI between fungicide programmes and N rates in Cycle II and Cycle III-B.

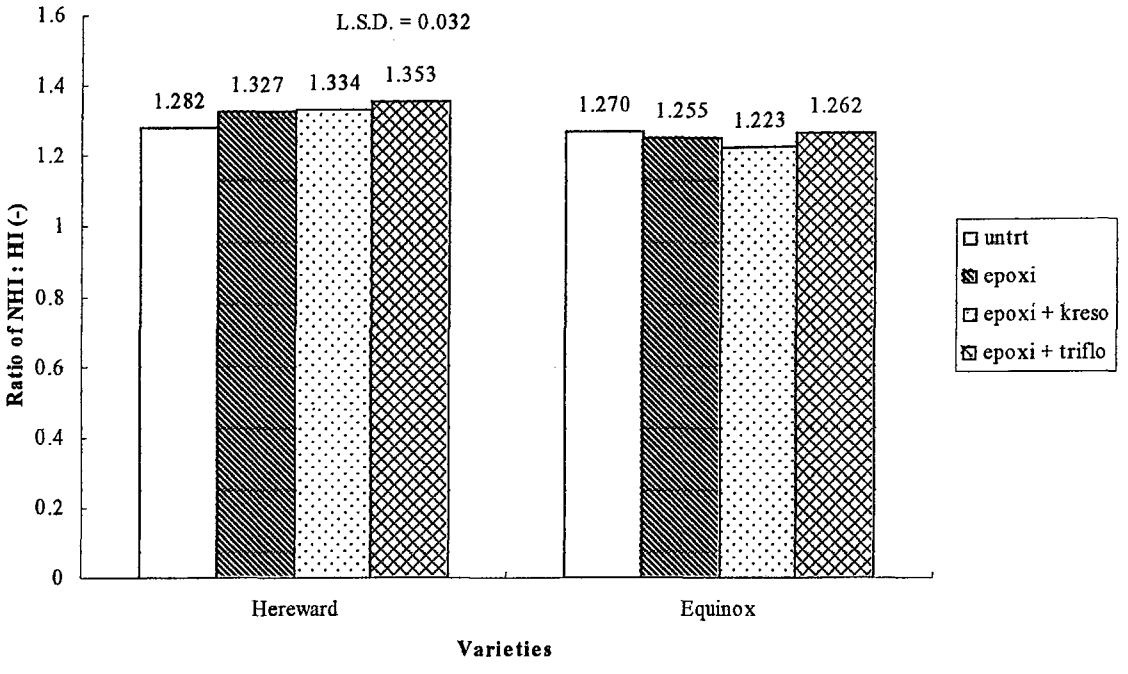


Figure 4.3.5.2 (a) The interaction in the ratio of NHI to HI between varieties and fungicide programmes at pre-harvest in Cycle III-C

untrt: untreated; epoxi: epoxiconazole; kreso: kresoxim-methyl; triflo: trifloxystrobin

Fungicide Programmes

In Cycle II, untreated plots and the plots treated with a mixture of epoxiconazole and trifloxystrobin showed a smaller ratio of NHI to HI than those treated with epoxiconazole alone by 0.015 and 0.020 respectively (P = 0.002) (Table 4.3.5.2 (a)) and than those treated with a mixture of epoxiconazole and kresoxim-methyl by 0.017 and 0.022 respectively (P = 0.002) (Table 4.3.5.2 (a)). In Cycle III-B, untreated plots showed a smaller ratio of NHI to HI than those treated with epoxiconazole alone and those treated with a mixture of epoxiconazole and trifloxystrobin by 0.042 and 0.058 respectively ($P < 0.001$) (Table 4.3.5.2 (a)). In Cycle III-C, the plots treated with a mixture of epoxiconazole and trifloxystrobin showed a greater ratio of NHI to HI than untreated plots and those treated with a mixture of epoxiconazole and kresoxim-methyl by 0.031 and

0.028 respectively ($P = 0.028$) (Table 4.3.5.2 (a)).

Table 4.3.5.2 (a) Ratio of NHI and HI of manually – harvested crops for fungicide programmes

Fungicide Programmes	*Cycle II*	*Cycle III-B*	*Cycle III-C*
untrt	1.392	1.372	1.276
epoxi	1.407	1.414	1.291
epoxi + kreso	1.409	-	1.279
epoxi + triflo	1.387	1.430	1.307
P value	= 0.002	< 0.001	= 0.028
L.S.D.	0.013	0.028	0.022
CV %	1.4	2.3	2.4

untrt: untreated; epoxi: epoxiconazole; kreso: kresoxim-methyl; triflo: trifloxystrobin

In Cycle II, there was no difference in ratio of NHI to HI between the two N rates tested in this field experiment (Table 4.3.5.2 (b)). In Cycle III-B, the plots that received no N showed a greater ratio of NHI to HI compared to those treated with the N rate of 100 kg ha^{-1} and that of 140 kg ha^{-1} by 0.146 and 0.172 respectively ($P < 0.001$) (Table 4.3.5.2 (b)). In Cycle III-C, the plots treated with the N rate of 100 kg ha^{-1} showed a greater ratio of NHI to HI than those treated with that of 140 kg ha^{-1} by 0.069 ($P < 0.001$) (Table 4.3.5.2 (b)).

Table 4.3.5.2 (b) Ratio of NHI and HI of manually – harvested crops for N rates

N rates ($kg\ ha^{-1}$)	*Cycle II*	*Cycle III-B*	*Cycle III-C*
0	-	1.511	-
90	1.402	-	-
100	-	1.365	1.323
130	1.396	-	-
140	-	1.339	-
200	-	-	1.254
P value	= 0.217	< 0.001	< 0.001
L.S.D.	NS	0.028	0.016
CV %	1.4	2.3	2.4

NHI was plotted against HI in Figure 4.3.5.2 (b). Both HI and NHI were relatively higher for the plots of Cycle II compared to those of Cycle III-B and Cycle III-C.

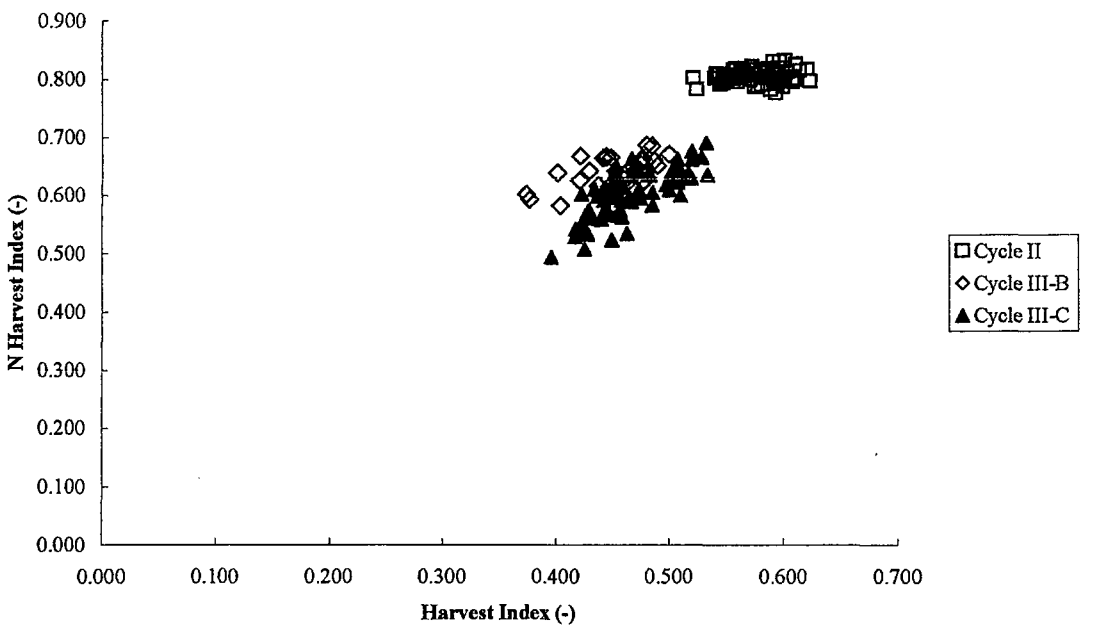


Figure 4.3.5.2 (b) The relationship between HI and NHI at pre-harvest

4.3.5.3 Single Grain N content (SGN)

No interaction between fungicide programmes and N rates was found in any of the four field experiments.

Fungicide Programmes

In Cycle I, the plots treated with a mixture of epoxiconazole and trifloxystrobin showed a greater SGN than untreated plots and the plots treated with epoxiconazole alone by 0.050 mg and 0.048 mg respectively (P = 0.049) (Table 4.3.5.3 (a)). In Cycle II, there was no difference in SGN between fungicide programmes (Table 4.3.5.3 (a)). In Cycle III-B, SGN was smaller for untreated plots than those treated with epoxiconazole alone and those treated with a mixture of epoxiconazole and trifloxystrobin by 0.05 mg and 0.07 mg respectively (P = 0.016) (Table 4.3.5.3 (a)). In Cycle III-C, untreated plots showed a smaller SGN than those treated with fungicides with the difference ranging from 0.108 mg to 0.142 mg ($P < 0.001$) (Table 4.3.5.3 (a)).

Table 4.3.5.3 (a) Single Grain N content (SGN) at pre-harvest for fungicide programmes

Fungicide Programmes	*Cycle I*	*Cycle II*	*Cycle III-B*	*Cycle III-C*
untrt	0.855	0.91	0.87	0.856
epoxi	0.857	0.91	0.92	0.964
epoxi + kreso	0.883	0.93	-	0.972
epoxi + triflo	0.905	0.88	0.94	0.998
P value	= 0.049	= 0.193	= 0.016	< 0.001
L.S.D.	0.040	NS	0.05	0.036
CV %	4.7	7.4	6.6	5.4

untrt: untreated; epoxi: epoxiconazole; kreso: kresoxim-methyl; triflo: trifloxystrobin

N rates

In Cycle I, Cycle II and Cycle III-C, the higher the N rate, the greater the SGN (Table 4.3.5.3 (b)). In Cycle III-B, SGN of the plots treated with the N rate of 140 kg ha^{-1} was significantly greater than untreated plots and those treated with the N rate of 100 kg ha^{-1} by 0.10 mg and 0.08 mg respectively ($P < 0.001$), however, the difference between untreated plots and those treated with the N rate of 100 kg ha^{-1} was not significant (Table 4.3.5.3 (b)).

Table 4.3.5.3 (b) Single Grain N content (SGN) at pre-harvest for N rates

N rates (kg ha^{-1})	*Cycle I*	*Cycle II*	*Cycle III-B*	*Cycle III-C*
0	0.784	-	0.87	-
90	-	0.88	-	-
100	0.882	-	0.89	0.898
130	-	0.94	-	-
140	0.959	-	0.97	-
200	-	-	-	0.997
P value	< 0.001	< 0.001	< 0.001	< 0.001
L.S.D.	0.035	0.03	0.05	0.026
CV %	4.7	7.4	6.6	5.4

4.3.6 N Accumulation Rate

4.3.6.1 The Time change in SGN

Cycle III-B

In Cycle III-B, there was no interaction in the rate of increase in SGN between fungicide programmes and N rates. The rate of increase in SGN was 3 to 4 times greater during the period from 258 days after sowing (DAS) to 272 DAS than that during the period from 272 DAS to 285 DAS (Fig. 4.3.6.1 (a), Fig. 4.3.6.1 (b), Table 4.3.6.1 (a), Table 4.3.6.1 (b)). There was no difference in the rate of increase in SGN between fungicide programmes during the period from 258 DAS to 272 DAS, however, untreated plots showed a smaller rate of increase in SGN than those treated with a mixture of epoxiconazole and trifloxystrobin during the period from 272 DAS and 285 DAS by 0.0034 mg day^{-1} (P = 0.045) (Table 4.3.6.1 (a)). During the period from 258 DAS and 272 DAS, the plots that received the N rate of 140 kg ha^{-1} showed a greater rate of increase in SGN than untreated plots and those that received the N rate of 100 kg ha^{-1} by 0.0032 mg day^{-1} and 0.0023 mg day^{-1} respectively (P = 0.021) (Table 4.3.6.1 (b)). There was no difference in the rate of increase in SGN between N rates during the period from 272 DAS to 285 DAS (Table 4.3.6.1 (b)).

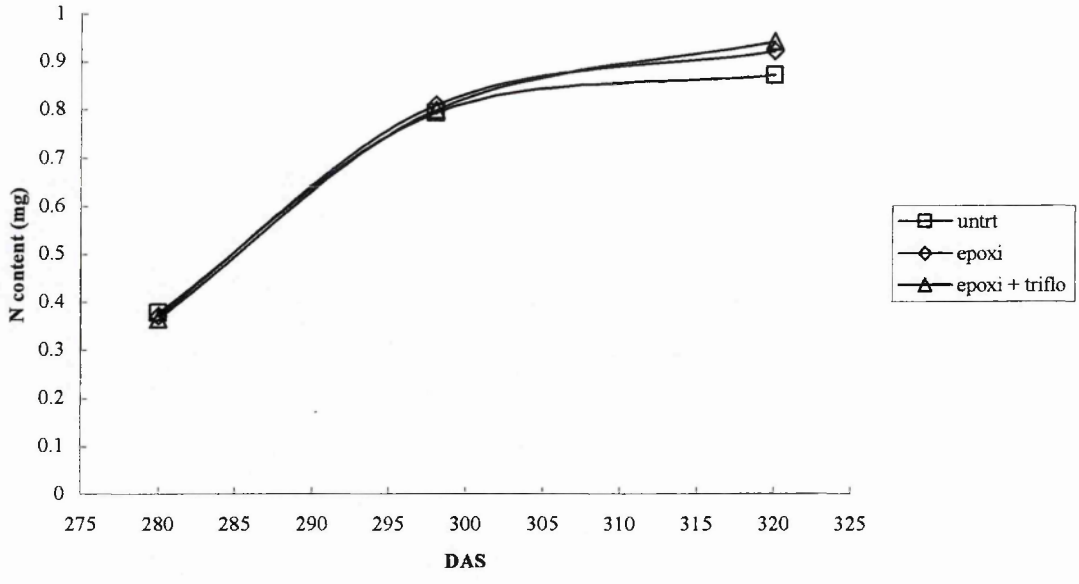


Figure 4.3.6.1 (a) The time change in single grain N content (SGN) for fungicide programmes during grain filling in Cycle III-B

untrt: untreated; epoxi: epoxiconazole; triflo: trifloxystrobin

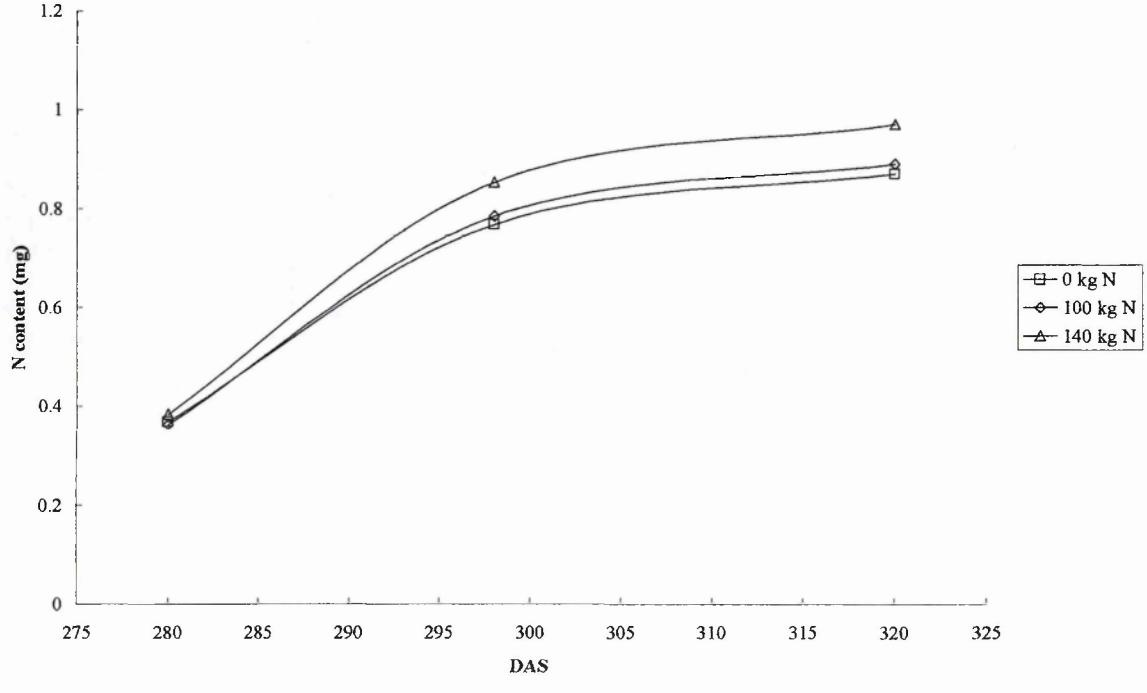


Figure 4.3.6.1 (b) The time change in single grain N content for N rates during grain filling in Cycle III-B

kg N: kg N ha^{-1}

Table 4.3.6.1 (a) The rate of increase in Single Grain N content (SGN) for fungicide programmes in Cycle III-B

Period	*Untrt*	*epoxi*	*epoxi + triflo*	*P value*	*LSD*	*CV %*
258 DAS - 272 DAS	0.0216	0.0236	0.0234	= 0.160	NS	11.5
272 DAS - 285 DAS	0.0036	0.0057	0.0070	= 0.045	0.0026	57.3

untrt: untreated; epoxi: epoxiconazole; triflo: trifloxystrobin

Table 4.3.6.1 (b) The rate of increase in Single Grain N content (SGN) for N rates in Cycle III-B

Period	*0 kg N*	*100 kg N*	*140 kg N*	*P value*	*LSD*	*CV %*
258 DAS - 272 DAS	0.0215	0.0224	0.0247	= 0.021	0.0022	11.5
272 DAS - 285 DAS	0.0052	0.0053	0.0059	= 0.846	NS	57.3

kg N: kg N ha^{-1}

Cycle III-C

In Cycle III-C, there was no interaction in the rate of increase in SGN between treatments. Equinox showed a greater rate of increase in SGN than Hereward by 0.0031 mg day^{-1} during the period from 302 DAS to 316 DAS (P = 0.009) (Table 4.3.6.1 (c)). Untreated plots showed a smaller rate of increase in SGN than the plots treated with fungicides by in the range of 0.0045 – 0.0067 mg day^{-1} during the same period ($P <$ 0.001) (Table 4.3.6.1 (d)). There was no difference in the rate of increase in SGN between the N rate of 100 kg ha^{-1} and that of 200 kg ha^{-1} during the period from 302 DAS to 316 DAS (Table 4.3.6.1 (e)).

Table 4.3.6.1 (c) The time change in Single Grain N content (SGN) for varieties in Cycle III-C

DAS	*Hereward*	*Equinox*	*P value*	*LSD*	*CV %*
302	0.912	0.830	< 0.001	0.022	4.9
316	0.968	0.927	= 0.003	0.026	5.4
dSGN ($mg\ d^{-1}$)	0.0039	0.0070	= 0.009	0.0022	80.7

Table 4.3.6.1 (d) The time change in Single Grain N content (SGN) for fungicide programmes in Cycle III-C

DAS	*untrt*	*epoxi*	*epoxi + kreso*	*epoxi + triflo*	*P value*	*LSD*	*CV %*
302	0.838	0.869	0.892	0.886	= 0.004	0.031	4.9
316	0.856	0.964	0.972	0.998	< 0.001	0.036	5.4
dSGN ($mg\ d^{-1}$)	0.0013	0.0067	0.0058	0.0080	< 0.001	0.0031	80.7

untrt: untreated; epoxi: epoxiconazole; kreso: kresoxim-methyl; triflo: trifloxystrobin

Table 4.3.6.1 (e) The time change in Single Grain N content (SGN) for N rates in Cycle III-C

DAS	*100 kg N*	*200 kg N*	*P value*	*LSD*	*CV %*
302	0.835	0.907	< 0.001	0.022	4.9
316	0.898	0.997	< 0.001	0.026	5.4
dSGN ($mg\ d^{-1}$)	0.0046	0.0063	= 0.110	NS	80.7

kg N: kg N ha^{-1}

4.3.6.2 The Time Change in Aboveground N Accumulation in Cycle III-B

An interaction in aboveground N accumulation rate between 4th (269 – 288 DAS) and 5th (295 – 302 DAS) samplings was observed between fungicide programmes and N rates. Although there was no difference in aboveground N accumulation rate between N rates for untreated plots and those treated with epoxiconazole

alone, among the plots treated with a mixture of epoxiconazole and trifloxystrobin, the plots treated with the N rate of 140 kg ha^{-1} showed a greater aboveground N accumulation rate than those treated with the N rate of 0 kg ha^{-1} and 100 kg ha-1 by 2.7 kg ha^{-1} day^{-1} and 2.2 kg ha^{-1} day^{-1} respectively (Fig. 4.3.6.2). When untreated plots and those treated with a mixture of epoxiconazole and trifloxystrobin were compared, with the N application rate of 140 kg ha^{-1}, aboveground N accumulation rate was greater for the plots treated with a mixture of epoxiconazole and trifloxystrobin than untreated plots by 2.2 kg ha^{-1} day^{-1}, while with no N application, it was greater for untreated plots than those treated with a mixture of epoxiconazole and trifloxystrobin by 2.2 kg ha^{-1} day^{-1} (Fig. 4.3.6.2).

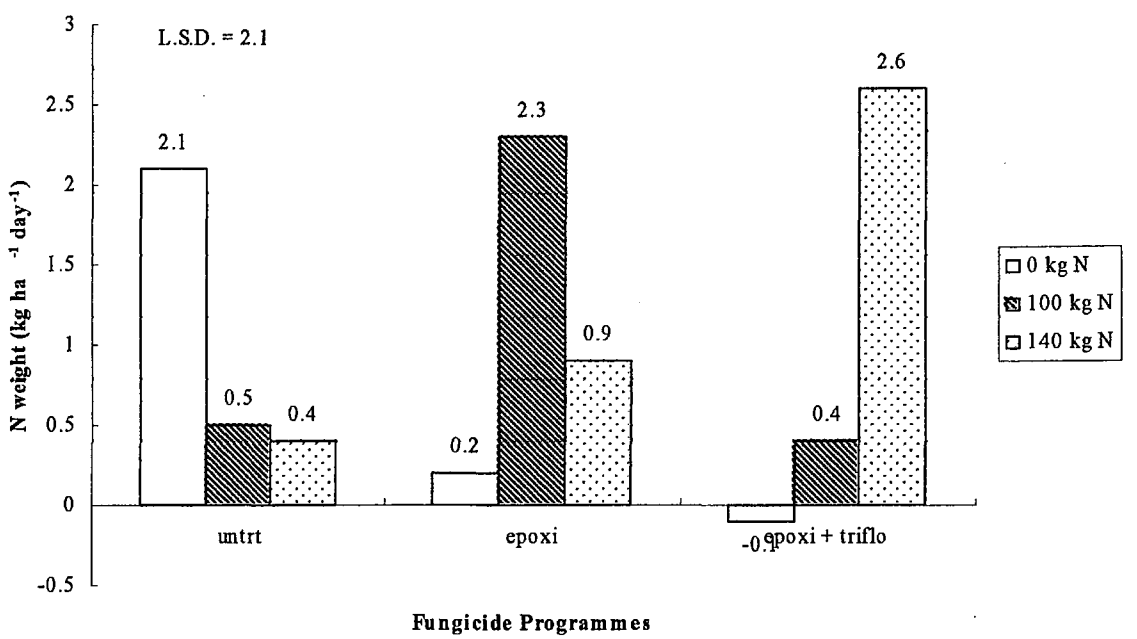


Figure 4.3.6.2 Aboveground N accumulation rate between the 4th (269 – 288 DAS) and the 5th (295 – 302 DAS) sampling in Cycle III-B

kg N: kg N ha^{-1}

In Cycle III-B, the rate of aboveground N accumulation did not show any difference between fungicide

programmes at any period between two consecutive samplings (Table 4.3.6.2 (a)). During the period between the 1st (194 – 198 DAS) and 2nd (237 – 244 DAS) sampling, the greater the N rate, the greater the rate of aboveground N accumulation ($P < 0.001$) (Table 4.3.6.2 (b)), however, no difference was observed between fungicide programmes and N rates during any period other than that (Table 4.3.6.2 (a), (b)).

Table 4.3.6.2 (a) The time change in aboveground N accumulation rate for fungicide programmes in Cycle III-B (kg ha^{-1} day^{-1})

Period (DAS)	*Untrt*	*epoxi*	*epoxi + triflo*	*P value*	*CV %*
(194-198) - (237-244)	1.82	1.81	2.06	= 0.180	18.8
(237-244)- (255-264)	0.5	0.9	0.7	= 0.534	133.5
(255-264) - (269-288)	1.6	0.9	1.3	= 0.431	106.9
(269-288) - (295-302)	1.0	1.2	1.0	= 0.942	138.7
(295-302) - (311-323)	-0.7	-0.3	-0.4	= 0.628	254.2

untrt: untreated; epoxi: epoxiconazole; triflo: trifloxystrobin

Table 4.3.6.2 (b) The time change in aboveground N accumulation rate for N rates in Cycle III-B (kg ha^{-1} day^{-1})

Period (DAS)	*0 kg N*	*100 kg N*	*140 kg N*	*P value*	*L.S.D.*	*CV %*
(194-198) - (237-244)	0.89	1.99	2.80	< 0.001	0.30	18.8
(237-244)- (255-264)	0.5	0.8	0.8	= 0.658	NS	133.5
(255-264) - (269-288)	1.6	1.3	0.8	= 0.324	NS	106.9
(269-288) - (295-302)	0.7	1.1	1.3	= 0.612	NS	138.7
(295-302) - (311-323)	-0.3	-0.1	-1.0	= 0.166	NS	254.2

kg N: kg N ha^{-1}

4.3.6.3 The Time Change in Grain N Accumulation in Cycle III-B

An interaction in grain N accumulation rate between 4th (269 – 288 DAS) and 5th (295 – 302 DAS) samplings was observed. Although the plots that received no N showed a smaller grain N accumulation rate between 4th (269 – 288 DAS) and 5th (295 – 302 DAS) samplings than those treated with fertilizer N for the plots treated with either epoxiconazole alone or a mixture of epoxiconazole and trifloxystrobin with the difference ranging from 1.7 to 2.8 kg ha^{-1} day^{-1}, there was no difference in grain N accumulation between N rates for untreated plots (Fig. 4.3.6.3). For the plots treated with a mixture of epoxiconazole and trifloxystrobin, the plots treated with the N rate of 140 kg ha^{-1} showed a greater grain N accumulation rate than those treated with that of 100 kg ha^{-1} by 1.1 kg ha^{-1} day^{-1} (Fig. 4.3.6.3). With no N application, untreated plots showed a greater grain N accumulation rate than those treated with epoxiconazole alone by 1.2 kg ha^{-1} day^{-1} (Fig. 4.3.6.3). With the N application rate of 140 kg ha^{-1}, the plots treated with epoxiconazole alone and those with a mixture of epoxiconazole and trifloxystrobin showed a greater grain N accumulation rate than

untreated plots by 1.1 kg ha^{-1} day^{-1} and 1.5 kg ha^{-1} day^{-1} respectively (Fig. 4.3.6.3).

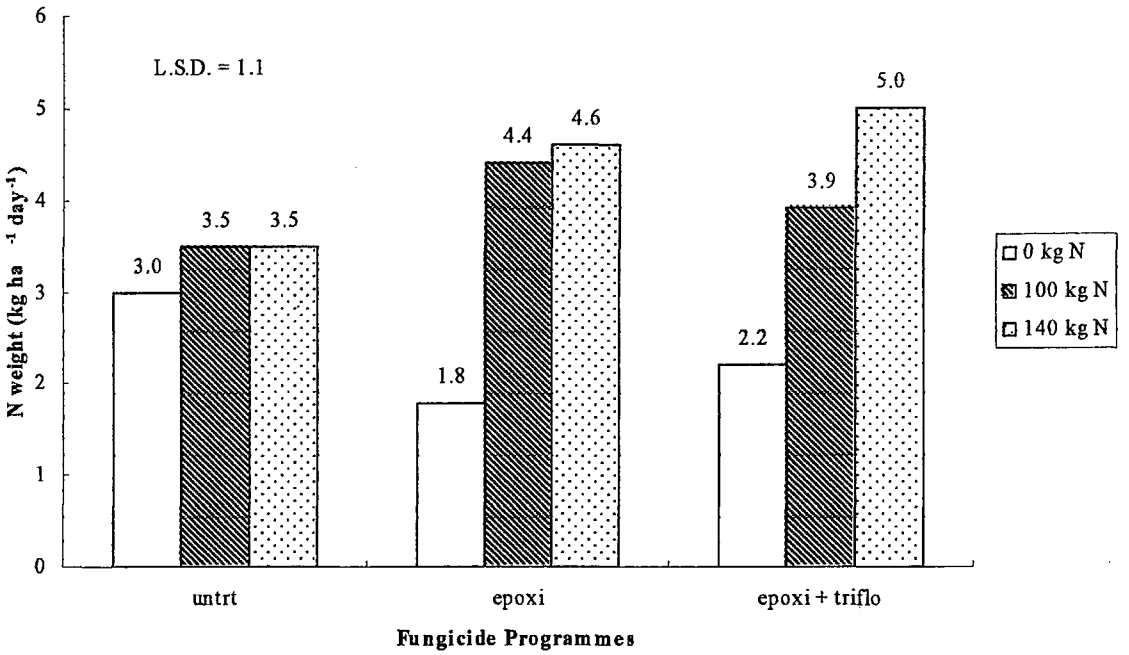


Figure 4.3.6.3 Grain N accumulation rate between the 4th (269 – 288 DAS) and 5th (295 – 302 DAS) sampling in Cycle III-B

untrt: untreated; epoxi: epoxiconazole; triflo: trifloxystrobin

kg N: kg N ha^{-1}

No difference was observed in the rate of grain N accumulation between fungicide programmes during any period between the consecutive samplings during grain-filling (Table 4.3.6.3 (a)). The plots treated with no N showed a smaller rate of grain N accumulation than those treated with the N rate of 100 kg ha^{-1} and 140 kg ha^{-1} by 1.6 kg ha^{-1} day^{-1} and 2.1 kg ha^{-1} day^{-1} respectively ($P < 0.001$) during the period between the 4th (269 – 288 DAS) and the 5th (295 – 302 DAS) sampling (Table 4.3.6.3 (b)).

Table 4.3.6.3 (a) The time change in grain N accumulation rate for fungicide programmes in Cycle III-B (kg ha^{-1} day^{-1})

Period (DAS)	*Untrt*	*epoxi*	*epoxi + triflo*	*P value*	*L.S.D.*	*CV %*
(269-288) - (295-302)	3.3	3.6	3.7	= 0.409	NS	21.6
(295-302) - (311-323)	0.3	0.7	0.9	= 0.235	NS	137.2

untrt: untreated; epoxi: epoxiconazole; triflo: trifloxystrobin

Table 4.3.6.3 (b) The time change in grain N accumulation rate for N rates in Cycle III-B (kg ha^{-1} day^{-1})

Period (DAS)	*0 kg N ha^{-1}*	*100 kg N ha^{-1}*	*140 kg N ha^{-1}*	*P value*	*L.S.D.*	*CV %*
(269-288) - (295-302)	2.3	3.9	4.4	< 0.001	0.7	21.6
(295-302) - (311-323)	0.3	0.9	0.6	= 0.178	NS	137.2

4.3.6.4 The Time Change in Vegetative N content in Cycle III-B

There was no interaction in the rate of vegetative N loss between fungicide programmes and N rates. There was no difference in the rate of vegetative N loss between fungicide programmes during any period between the consecutive samplings during grain-filling (Table 6.3.6.4 (a)). During the period between the 4th (269 – 288 DAS) and the 5th (295 – 302 DAS) sampling, the plots that received no N showed a smaller rate of vegetative N loss than the plots treated with the N rate of 100 kg ha^{-1} and 140 kg ha^{-1} by 1.3 kg ha^{-1} day^{-1} and 1.5 kg ha^{-1} day^{-1} respectively (P = 0.002) (Table 4.3.6.4 (b)). Between the 5th and the 6th sampling, the plots treated with the N rate of 140 kg ha^{-1} showed a greater rate of vegetative N loss than untreated plots and those treated with the N rate of 100 kg ha^{-1} by 1.05 kg ha^{-1} day^{-1} and 0.57 kg ha^{-1} day^{-1} respectively (P < 0.001) (Table 4.3.6.4 (b)).

Table 4.3.6.4 (a) The time change in vegetative N loss rate for fungicide programmes in Cycle III-B (kg ha^{-1} day^{-1})

Period (DAS)	*Untrt*	*epoxi*	*epoxi + triflo*	*P value*	*L.S.D.*	*CV %*
(269-288) - (295-302)	2.3	2.5	2.8	= 0.532	NS	38.2
(295-302) - (311-323)	1.00	0.89	1.31	= 0.197	NS	53.1

untrt: untreated; epoxi: epoxiconazole; triflo: trifloxystrobin

Table 4.3.6.4 (b) The time change in vegetative N loss rate for N rates in Cycle III-B (kg ha^{-1} day^{-1})

Period (DAS)	*0 kg N ha^{-1}*	*100 kg N ha^{-1}*	*140 kg N ha^{-1}*	*P value*	*L.S.D.*	*CV %*
(269-288) - (295-302)	1.6	2.9	3.1	= 0.002	0.8	38.2
(295-302) - (311-323)	0.56	1.04	1.61	< 0.001	0.5	53.1

4.3.7 Apparent Recovery of Fertilizer N at Pre-Harvest

Both in Cycle I and Cycle III-B, there was no interaction between fungicide programmes and N rates in apparent recovery of fertilizer N (Table 4.3.7). Both in Cycle I and Cycle III-B, no difference in apparent recovery of fertilizer N was observed between either fungicide programmes or N rates, although the differences between untreated plots and those treated with fungicides in Cycle III-B were close to significant ($P = 0.063$) (Table 4.3.7).

Table 4.3.7 Apparent Recovery of Fertilizer N in Cycle I and Cycle III-B (%)

Field Experiment	*Cycle I*		*Cycle III-B*	
Fungicides/N rates	***100 kg N***	***140 kg N***	***100 kg N***	***140 kg N***
Untrt	112	118	84	76
Epoxi	99	110	120	98
epoxi + kreso	91	96	-	-
epoxi + triflo	105	105	121	108
P value (Fungicides)	= 0.318		= 0.063	
P value (N rates)	= 0.511		= 0.246	
P value (Interaction)	= 0.895		= 0.871	
CV %	18.2		28.3	

untrt: untreated; epoxi: epoxiconazole; kreso: kresoxim-methyl; triflo: trifloxystrobin

kg N: kg N ha^{-1}

4.3.8 Soil Mineral N in Cycle I

Soil mineral N was found to be higher after harvest than in spring for most of the plots (Table 4.3.8 (a)). No interaction was observed in soil mineral N between fungicide programmes and N rates. The plots treated with a mixture of epoxiconazole and trifloxystrobin showed a lower soil mineral N after harvest than untreated plots and those treated with epoxiconazole alone by 13 kg ha^{-1} and 9 kg ha^{-1} respectively (P = 0.011) (Table 4.3.8 (b)). Soil mineral N after harvest was greater for untreated plots than the plots treated with a mixture of epoxiconazole and kresoxim-methyl by 7 kg ha^{-1} ($P < 0.011$). The plots treated with the N rate of 100 kg ha^{-1} showed a lower soil mineral N than the plots that received no N by 8 kg ha^{-1} ($P = 0.046$) (Table 4.3.8 (c)). These were still significant when soil mineral N in spring was added as a covariant (P = 0.014). The difference in soil mineral N between the samples taken in March and those in September was not significantly different for different treatments (data not shown).

Table 4.3.8 (a) Means of soil mineral N of 90 cm depth for each treatment in spring and after harvest in Cycle I (kg ha^{-1})

Treatment (Fungicides - N rates)	*Spring*	*After harvest*
untrt-0 kg N	38	68
untrt-100 kg N	34	55
epoxi-0 kg N	35	65
epoxi-100 kg N	37	54
epoxi-140 kg N	43	56
epoxi + kreso-0 kg N	41	56
epoxi + kreso-100 kg N	49	53
epoxi + kreso-140 kg N	42	56
epoxi + triflo-0 kg N	36	52
epoxi + triflo-100 kg N	27	46
epoxi + triflo-140 kg N	34	50
S.E.	8	4
CV %	38.6	13.1

untrt: untreated; epoxi: epoxiconazole; kreso: kresoxim-methyl; triflo: trifloxystrobin

kg N: kg N h^{-1}

Table 4.3.8 (b) Soil Mineral N of 90 cm depth for fungicide programmes after Harvest in Cycle I

Fungicide Programmes	*Soil Mineral N (kg ha^{-1})*
Untrt	62
Epoxi	58
epoxi + kreso	55
epoxi + triflo	49
P value	= 0.011
L.S.D.	7
CV %	13.1

untrt: untreated; epoxi: epoxiconazole; kreso: kresoxim-methyl; triflo: trifloxystrobin

Table 4.3.8 (c) Soil Mineral N of 90 cm depth for N rates after Harvest in Cycle I

N rates (kg ha^{-1})	*Soil Mineral N (kg ha^{-1})*
0	60
100	52
140	56
P value	= 0.046
L.S.D.	6
CV %	13.1

4.4 Discussion

4.4.1 N content

No interactive effects were observed between fungicide programmes and N rates on aboveground N content in any of the field experiments conducted in this study. Fungicide programmes did not affect aboveground N content at pre-harvest, apart from Cycle III-C where the plots treated with fungicides showed a significantly greater aboveground N content than untreated plots most likely due to a greater severity of *Septoria* diseases observed with untreated plots in this field experiment (Black, 2003). An increased N uptake of strobilurin-treated wheat was reported by Bryson (2000), while a case was reported where strobilurin fungicides did not cause any increase in N uptake (Jones *et al.*, 2001).

As to grain N content, however, synergistic effects of fungicide programmes and N rates were observed. In Cycle III-B, the N application rate of 140 kg ha^{-1} significantly increased the grain N content compared to that of 100 kg ha^{-1} only for the plots treated with fungicides, indicating a positive synergistic interaction between fungicide programmes and N rates as to grain N accumulation. Apart from Cycle II, grain N content was always found to be greater, at least numerically, for the plots treated with a combination of triazole and strobilurin, a mixture of epoxiconazole and trifloxystrobin than untreated plots and those treated with either triazole alone, epoxiconazole, or the other combination of triazole and strobilurin, a mixture of epoxiconazole and kresoxim-methyl. When limited to the case of combine-harvested grains, grain N content of the plots treated with a mixture of epoxiconazole and trifloxystrobin was always, and significantly, greater than that of

the plots treated with either no or other fungicide programmes. There was no single observation that showed a significantly greater grain N content of the plots treated with the other combination of triazole and strobilurin, a mixture of epoxiconazole and kresoxim-methyl. The mechanism as to how the fungicide programme of a mixture of epoxiconazole and trifloxystrobin influenced the crop to accumulate greater N in grains is not known. It might be simply the consequence of different performance between the two strobilurin fungicide programmes observed in this study as to the maintenance of green leaf area considering that the plots treated with a mixture of epoxiconazole and trifloxystrobin showed a significantly greater DMW of green leaf as well as LAI compared to those treated with a mixture of epoxiconazole and kresoxim-methyl. Maintenance of green canopy for a longer period might enable the crop to take up more N as indicated by Ottman and Welch (1988). However, a great care must be taken to make such a conclusion, as in this study the number of observations made on the crops treated with a mixture of epoxiconazole and kresoxim-methyl was not sufficient with respect to the maintenance of green canopy. A possibility remains that the two strobilurin fungicide programmes might affect the process of grain N accumulation differently.

A varietal difference was observed between Hereward and Equinox in grain N content. In Cycle III-C, Equinox and Hereward responded differently to a mixture of epoxiconazole and trifloxystrobin in regard of grain N content. Bayles (1999) reported Equinox to be a more responsive variety to kresoxim-methyl compared to Hereward with respect to grain yield. It was, however, not known whether such a difference in yield response between fungicide programmes was accompanied by a proportional difference in plant and

grain N accumulation. In Cycle III-C of this study, neither Hereward nor Equinox responded to the fungicide programme of a mixture of epoxiconazole and kresoxim-methyl any differently to that of epoxiconazole alone. Equinox, however, showed a significantly greater grain N content in response to the fungicide programme of a mixture of epoxiconazole and trifloxystrobin compared to the other two fungicide programmes, i.e. epoxiconazole alone and a mixture of epoxiconazole and kresoxim-methyl. Based on the observations in this study so far as to grain N content, the dilution of grain N concentration appears more likely to occur with the crops treated with a mixture of epoxiconazole and kresoxim-methyl than those treated with a mixture of epoxiconazole and trifloxystrobin.

As to N rates, for any of the field experiments, both aboveground N content and grain N content were greater when treated with a greater N rate when compared within each experiment. In Cycle I, aboveground N content increased by 19 kg ha^{-1} for the plots treated with the N rate of 140 kg ha^{-1} during the period between 272 and 285 days after sowing, while there was hardly any change for the plots that received either no or the N rate of 100 kg ha^{-1}. It is interesting to note that the difference in the rate of fertilizer N application as early as March and April time caused such a difference in the pattern of N uptake during grain filling period. It is expected that N uptake during grain filling period would increase grain N content rather than yield as reported by others (Finney *et al.*, 1957; Spiertz and De Vos, 1983).

4.4.2 N Harvest Index (NHI)

In Cycle II, a slight improvement of NHI was observed with the N application rate of 130 kg ha^{-1} for the plots

treated with a mixture of epoxiconazole and trifloxystrobin compared to untreated plots. Such an interaction between fungicide programmes and N rates was not observed in other field experiments. It is not possible to interpret the interaction from this single observation. Besides, Cycle II was conducted in a 'strange weather' year and the crops grown under such a condition may be different in some aspects from those in other field experiments. In Cycle III-C, the NHI of Equinox was significantly increased by the application of a mixture of epoxiconazole and trifloxystrobin compared to that of a mixture of epoxiconazole and kresoxim-methyl, while such an effect was not observed for Hereward.

As to overall effects of fungicides on NHI, application of fungicides tended to improve NHI in Cycle III-B and Cycle III-C, which agrees to the observation by Leitch and Jenkins (1995) that premature senescence caused by *Septoria* diseases trapped N in the senesced leaves. The degree of improvement was greater for the plots treated with a mixture of epoxiconazole and trifloxystrobin implying an improved efficiency in translocation of N for the plots treated with this fungicide programme, however, this was not always statistically significant. The difference in NHI between fungicide programmes might only be reflecting the difference in the degree of control over *Septoria* diseases by fungicide programmes. In order to test the hypothesis whether NHI is affected by different fungicide programmes as the consequence of physiological change rather than that of different degrees in disease control, desirably NHI should be tested in a field experiment in the absence of foliar diseases, however, such an experiment is very difficult, if not impossible, in the field. The difference in NHI between untreated plots and those treated with fungicides seem to have occurred during the later phase of grain fill, which supports the view by Leitch and Jenkins (1995). King

(2002) observed a single case where NHI was slightly lower for the plots treated with a mixture of epoxiconazole and azoxystrobin compared to those treated with either a mixture of epoxiconazole and morpholine or a mixture of epoxiconazole and trifloxystrobin.

Interestingly NHI was hardly influenced by different N rates apart from Cycle III-C where the plots treated with the N rate of 100 kg ha^{-1} showed a significantly greater NHI than those treated with that of 200 kg ha^{-1}, a decreased efficiency in N translocation. It could be inferred that the rate of N application, at least applied at GS 30/31 and GS 39, is not likely to affect NHI except when supraoptimum N is applied, which was probably the case in Cycle III-C.

4.4.3 N Partitioning

In Cycle II, the plots treated with a combination of triazole and strobilurin, a mixture of epoxiconazole and kresoxim-methyl, tended to contain a greater N content of lower part of canopy both in stem and leaf around anthesis when treated with a higher rate of N (130 kg ha^{-1}). Such an interaction between fungicide programmes and N rates, however, was not observed at pre-harvest/harvest. Both in Cycle I and Cycle III-B, N content of green leaf was kept greater and that of senesced leaf lower when fungicides were applied especially a combination of triazole and strobilurin, a mixture of epoxiconazole and trifloxystrobin, implying a greater photosynthetic ability of green leaf and more efficient extraction of N from senesced leaf of the plots treated with a mixture of epoxiconazole and trifloxystrobin compared to untreated plots, the plots treated with triazole alone and those treated with a mixture of epoxiconazole and, in the case of Cycle I,

kresoxim-methyl, another combination of triazole and strobilurin.

There appears to be complex mechanisms of interactions between environment and the way a given fungicide programme affects the crop even between the different chemistries of the same fungicide group. Plotting leaf N content against LAI gave graphs that help us to understand what was happening with partitioning of N in the canopy as a whole. It is observed from these graphs that the plots treated with lower N rates tended to lose N from lower part of the canopy faster than those treated with higher N rates, while fungicide programmes appear to have more to do with the N content of a canopy as a whole.

In Cycle III-B, Specific Leaf N (SLN) was compared between fungicide programmes around the time of maximum canopy. There was no statistically significant difference in SLN between untreated plots and the plots treated with epoxiconazole alone, while there was between untreated plots and the plots treated with a mixture of epoxiconazole and trifloxystrobin. Considering that the difference in SLN could amount to the difference in CO_2 exchange rate and consequently RUE (Sinclair and Horie, 1989), the advantage of the plots treated with a mixture of epoxiconazole and trifloxystrobin over untreated plots in carbon assimilation is suspected.

4.4.4 Grain N concentration

Plotted against grain yield, the distribution of grain N concentration of manually-harvested grains at pre-harvest was more scattered than that of combine-harvested grains, in other words, it was characterized

with a greater CV. Apart from the fact that the number of samples that could be taken was much smaller for manual-harvest than combine-harvest, therefore, giving a greater CV for manual-harvested grains, the nature of the two methods of harvest should be considered. Smaller grains tend to escape when combine-harvested. Therefore, the difference in profile of sampled grains might have contributed to the difference in grain N concentration of the two methods of harvest. Higher grain N concentration observed with untreated plots of Equinox in Cycle III-C is considered to be due to a higher level of *Septoria* diseases observed with this treatment in this field experiment, as a reduction in TGW is known to be caused by *Septoria* diseases (Simon *et al.*, 2002).

N concentration of vegetative parts was found to be lower for the plots treated with fungicides than untreated plots implying improved efficiency in translocation of N following the application of fungicides. In Cycle III-C, N concentration of vegetative parts was lower for the plots treated with a mixture of epoxiconazole and trifloxystrobin than for those treated with a mixture of epoxiconazole and kresoxim-methyl indicating different efficiencies in the translocation of N from vegetative parts to grains between the two fungicide programmes. Despite both being the combinations of triazole and strobilurin fungicides, they seem to act differently to each other with respect to the translocation of C and N. N concentration of aboveground plant at pre-harvest varied greatly between field experiments, showing a particularly lower level for Cycle I compared to other field experiments. Where the highest N rate (i.e. 140 kg ha^{-1}) was applied, 2 plots out of 3 met the requirement of grain N concentration for bread-making both for the plots treated with epoxiconazole alone and those treated with a mixture of epoxiconazole and trifloxystrobin. It was only 1

plot out of 3 in the case of the plots treated with a mixture of epoxiconazole and kresoxim-methyl. This result, however, was derived from manually-harvested grains, which does not happen in reality.

Grain N concentration of combine-harvested grains was lower than that and in Cycle I there was no single plot that met the requirement. N fertilization was obviously not enough to achieve the quality for bread-making in Cycle I even the plots treated with the highest N rate (i.e. 140 kg ha^{-1}) in this experiment. In Cycle II, more than 2/3 of the plots achieved the requirement irrespective of the fungicide programmes in the case of Hereward, a bread-making variety. Overall the performance of Malacca was not as good as that of Hereward, however, more than 2/3 of the plots achieved the requirement as well where the highest N rate (i.e. 130 kg ha^{-1}) was applied. In the case of Equinox, grain N concentration was lower than the requirement for bread-making apart from a few exceptions confirming that grain N concentration is controlled by genetic factor. Cycle III-B was even worse than Cycle I with respect to grain N concentration in the context of bread-making. For manual-harvest, only 1 out of 4 plots met the requirement irrespective of fungicide programmes, not mentioning only 1 plot qualified for bread-making out of 36 plots for combine-harvest. The situation was much better in Cycle III-C where all the plots of Hereward treated with the highest N rate (i.e. 200 kg ha^{-1}) achieved the requirement in the case of combine-harvest. Similarly to Cycle II, grain N concentration of Equinox hardly exceeded the requirement for bread-making. The plots that exceeded are considered to be those characterized with exceptionally severe level of *Septoria* diseases (Black, 2003) likely to be caused by a significant reduction in TGW.

As to varieties, neither between untreated plots and the plots treated with fungicides nor between those treated with different fungicide programmes was there a significant difference in grain N concentration for Hereward, while for Equinox untreated plots showed a greater grain N concentration than the plots treated with fungicides. The plots treated with a combination of triazole and strobilurin, a mixture of epoxiconazole and kresoxim-methyl, showed a significantly lower N concentration of combine-harvested grains than those treated with triazole alone, which might indicate a dilution of grain N considering that there was no significant difference in grain N content between the plots treated with the two fungicide programmes. Grain N concentration was always increased by the increased rates of N fertilizer in any of the field experiments in this study.

4.4.5 Plant N concentration

In Cycle III-B and Cycle III-C the N concentration of vegetative parts at pre-harvest was observed to be significantly greater for untreated plots than the plots treated with fungicides indicating that a greater portion of N was kept in vegetative parts for untreated plots. In Cycle III-C, a significant difference was observed in the N concentration of vegetative parts between the plots treated with a mixture of epoxiconazole and kresoxim-methyl and those treated with a mixture of epoxiconazole and trifloxystrobin, with the former showing a greater N concentration than the latter, which seems to have caused a lower grain N concentration observed for the latter as discussed in the previous section. Viewed at aboveground plant basis, N concentration was found to be relatively lower in Cycle I compared to that in Cycle III-B with the magnitude of the difference being as great as 40 %, despite that both of the field experiments received exactly the same

rates of N application. Although yield was relatively greater in Cycle I than that in Cycle III-B, the yield difference alone does not seem to provide sufficient explanation. It looks that the rest of the information would have to come from factors of soil and environment.

4.4.6 N accumulation in relation to DM accumulation

Pearson's correlation analysis between traits related to DM and N accumulations in Cycle II (*cv* Hereward) and Cycle III-B revealed a contrasting nature of these two field experiments. A positive correlation was observed between grain yield and grain N concentration in Cycle III-B, while there was no correlation between the two traits in Cycle II. In Cycle III-B, grain yield, grain N concentration and grain N yield were found to be positively correlated with Harvest Index. In Cycle II, on the other hand, grain yield and grain N concentration were found to be negatively and positively correlated with N Harvest Index respectively. In both of the field experiments, HI and NHI were positively correlated. From these, it could be inferred that the factor of DM accumulation in grains played a dominant role in determining grain N concentration in Cycle III-B, while N accumulation was more dominant in Cycle II. The ratio of NHI to HI was used to study the relative dominance of translocation between C and N. Interestingly, the ratio of NHI to HI was influenced by fungicide programmes in all the three field experiments where the ratio was studied (i.e. Cycle I, Cycle II and Cycle III-B). Untreated plots tended to show a smaller ratio of NHI to HI compared to those treated with fungicides implying a relative reduction in N translocation, which appears to be somewhat peculiar considering that a greater grain N concentration was observed with untreated plots than those treated with fungicides. It is because both HI and NHI are relative indexes which do not contain the information as

to the size of the pool of C and N respectively, while grain N concentration is determined by the relativity of the two pools (i.e. C and N) of absolute value. The implication is that where no fungicides are applied, the translocation process of N is likely to be more damaged than that of C, DM, however, as the reduction in the size of the pool of C is greater than that of N, grain N concentration becomes greater. The logic seems to agree to the observation made by Leitch and Jenkins (1995) that premature senescence caused by *Septoria* diseases trapped N in the senesced leaves, obviously reducing NHI. Contrary to untreated plots, the ratio of NHI to HI tended to be greater for the plots treated with a mixture of epoxiconazole and trifloxystrobin indicating a relative dominance of the translocation of N to that of C except in Cycle II that cannot seem to be directly compared to the experiments conducted on a 'standard crop'.

Single Grain N content (SGN) revealed a tendency that a greater amount of N was available to each grain when treated with fungicides, which again seems to support the above view that the translocation process of N is likely to be more damaged than that of C when no fungicides are applied. In Cycle I, SGN was greater for the plots treated with a mixture of epoxiconazole and trifloxystrobin compared to those treated with epoxiconazole alone, indicating some advantage of using the combination of triazole and strobilurin in obtaining greater N accumulation.

4.4.7 N accumulation rate

The rate of N accumulation was observed at single grain basis and unit of land basis. At single grain basis, in neither Cycle III-B nor Cycle III-C was there interactions in the rate of N accumulation between fungicide

programmes and N rates. The rate of N accumulation was significantly greater for the plots treated with fungicides than untreated plots both in Cycle III-B and Cycle III-C, although it seemed to have been the case only during the later phase of grain fill rather than the early phase. Contrary to fungicide programmes, it was during the early phase of grain fill than the later phase that greater rates of N application significantly favoured the rate of N accumulation in single grain than lower rates. These observations may imply that both fungicide programmes and N rates are likely to affect the process of grain filling but in different phases.

At unit of land basis, the rate of grain N accumulation was interactively influenced by fungicide programmes in Cycle III-B. For the plots treated with triazole alone, it was greater for the plots treated with either the N rate of 100 kg ha^{-1} or that of 140 kg ha^{-1} compared to untreated plots, while for the plots treated with a combination of epoxiconazole and trifloxystrobin, the greater the N rate, the significantly greater the rate of grain N accumulation. When averaged over N application rates, there was no difference in the rate of grain N accumulation between fungicide programmes. On the other hand, N application rates caused a difference in the rate of grain N accumulation that the greater the N rate, the greater the rate of grain N accumulation. It was the case during the early phase of grain fill but not during the later phase, similarly to the observation made on the accumulation rate of single grain N.

The rate of aboveground N accumulation was interactively affected by fungicide programmes and N rates. For the plots treated with triazole alone, a greater rate of aboveground N accumulation was observed for the plots treated with the N rate of 100 kg ha^{-1} than untreated plots. For the plots treated with a combination of

triazole and strobilurin, a mixture of epoxiconazole and trifloxystrobin, the plots treated with the N rate of 140 kg ha^{-1} showed a significantly greater rate of aboveground N accumulation than untreated plots and those treated with the N rate of 100 kg ha^{-1}. These observations may indicate a different way of taking up N from soils between the plots treated with triazole alone and those treated with a combination of triazole and strobilurin, although a further investigation would be needed for confirmation. The rate of aboveground N accumulation was influenced by N rates only the period commencing immediately after the second application of N fertilizer. There was no evidence from Cycle III-B that fungicide programmes affect the rate of N loss from vegetative parts, while there were effects of N rates on the rate of N loss from vegetative parts. The plots treated with a greater rate of N tended to show a greater rate of N loss from vegetative parts.

4.4.8 Soil Mineral N

Taking soil cores from 90 cm depth is thought to be sufficient for the purpose of making N fertilizer recommendations in early spring, as rooting density below 90 cm are not likely to be significant enough to be considered (Kuhlmann *et al.*, 1989). The level of mineral soil N was greater when measured in early spring before the N application at GS 30/31 than after harvest probably due to a greater rate of mineralization of organic N enhanced by higher temperature during summer. Soil mineral N after harvest was significantly lower for the plots treated with a combination of triazole and strobilurin, a mixture of epoxiconazole and trifloxystrobin than untreated plots and those treated with triazole alone indicating an increased uptake by the crop. The plots treated with the other combination of triazole and strobilurin, a mixture of epoxiconazole

and kresoxim-methyl showed a significantly lower soil mineral N than untreated plots. In both cases, however, no increase in aboveground N content was observed at pre-harvest even though there remains a slight possibility of a further N uptake by the crop after pre-harvest sampling considering that there were almost three weeks interval between pre-harvest sampling and combine-harvesting in this field experiment. There have been reports on increased N uptake by the strobilurin-treated wheat crop (Bryson, 2000; Anon., 2003a), but at the same time other workers have claimed that the N requirement of strobilurin-treated crops is similar to triazole-treated crops (Clark and Jones, 1999; Jones *et al.*, 2001). The observation made on mineral soil N with the plots treated with a mixture of epoxiconazole and trifloxystrobin in Cycle I of this study seem to support the former, while the lack of difference in aboveground N uptake between fungicide programmes at pre-harvest of Cycle II, Cycle III-B and Cycle III-C agrees with the latter. Even though a possibility of increased N uptake following the application of strobilurin fungicide programmes cannot be excluded, this study seemed to have shown that such an effect of strobilurin fungicide programmes on crop N uptake is not a consistent event.

Chapter 5

Bioregulatory Effects of Strobilurin Fungicides

5.1 Introduction

5.1.1 Phylloplane Fungi

Dickinson and Wallace (1976) noted that despite an important role which fungicides play in the control of plant diseases, their effects against organisms other than the target pathogen and its host are not known. Fungicides of a broad spectrum such as zineb and benomyl have been reported to be effective against a wide range of phylloplane saprophytes (Dickinson, 1973). It is known that plant senescence could be accelerated by some phylloplane fungi (Skidmore and Dickinson, 1973; Jachmann and Fehrmann, 1989), which is sometimes observed as prolonged leaf life following fungicide spraying (Dickinson, 1973). Fokkema and De Nooij (1981) pointed out the possibility that the pathogens that are not sensitive, or those which have acquired resistance to the fungicides applied, will be favoured by the reduction of the saprophytic mycoflora following fungicide application. The strobilurins show a wide spectrum of disease control and therefore are expected to affect phylloplane fungi as other fungicides.

5.1.2 Plant hormones

Grossmann and Retzlaff (1997) placed wheat leaf discs in Petri dishes containing kresoxim-methyl and found that ethylene formation was reduced by up to 40 % after exposure for 24 hours. Ethylene is a plant

hormone functioning as a potent growth regulator, affecting the growth, differentiation, and senescence of plants, in concentrations as little as 0.01 μ l litre^{-1} (Reid, 1995). As ethylene enhances leaf aging and senescence, this might suggest that the inhibition of ethylene synthesis caused by kresoxim-methyl is related to its senescence-delaying activity. Furthermore, Grossman *et al.* (1999) observed up to two-fold increase in endogenous levels of abscisic acid (ABA) compared to control following foliar-treatment of kresoxim-methyl. At the same time, the efficiency of water consumption by the treated plants was improved. ABA is known to close stomata in response to water stress (Taiz and Zeiger, 1998). Grossman *et al.* (1999), therefore, considered that kresoxim-methyl increased ABA and consequently improved water efficiency. Investigation on the effects of the strobilurins on plant hormones is still limited to kresoxim-methyl.

5.1.3 Morphology

It is known that application of some chemicals affect plant morphology. Izumi *et al.* (1984) found a decreased level of gibberellins in shoots following the application of a dwarfing agent of triazole group, uniconazole. Benton and Cobb (1995) treated *Galium aparine* L. with 125 g ai ha^{-1} of epoxiconazole and observed at 7 days after the treatment that the treated plants grew to a lesser height by as great as 43 %. Further they observed a reduction in leaf area without altering leaf fresh weight. Microscope examination revealed that palisade, spongy mesophyll and upper epidermal cells were increased in size following the application of epoxiconazole resulting in thicker leaves. The consequence of such a modification in plant morphology may not be small. Sumi and Katayama (2001) grew tall and dwarf isogenic lines of sorghum,

soybean and rice and found that the dwarf lines tended to use less water to achieve the same level of the yield as the tall lines indicating improved characteristics of the dwarf lines in water use efficiency and consequently in avoidance of drought injury. This may suggest that the plants treated with a dwarfing agent such as growth regulator may achieve improved water use efficiency in the similar manner as dwarf isogenic lines.

5.1.4 Nutrient Leaching by Rainfall Exposure

A great diversity of materials both inorganic and organic substances has been reported to leach from plants (Wetselaar and Farquhar, 1980), for example, by exposure to rainfall (Tukey, 1970; Kimura and Ariyoshi, 1994). Tanakamaru *et al.* (1998) conducted a glasshouse experiment exposing two near-isogenic lines of barley; one is normal and the other is characterized with "glossy" leaf to rainfall to compare wettability between the two lines. The amount of epicuticular wax in leaves of the glossy line was almost half that of those of the normal line. Exposure to rainfall reduced the amount of epicuticular wax in both lines but the extent of the reduction was greater in the glossy line than the normal line. At the same time it was observed that older leaves tended to lose epicuticular wax to a greater extent compared to younger leaves. Although they could not clarify the mechanism of wax reduction in leaves following rainfall exposure, they suggested that the degree of wax reduction was closely related to leaf wettability. An implication could be made here that exposure not only to rainfall but also to chemical solutions such as fungicides may alter wax content of leaves, possibly affecting the leaf life as well as susceptibility to nutrient leaching and also composition of phylloplane fungi. From this point of view, it may be interesting to study the effects of applying fungicides

on the state and property of plant surface.

5.1.5 Objectives and Hypothesis of Chapter 5

This chapter deals with the objective (IV) in the section of '*1.2 Aim, Objectives and Approach*' of Chapter 1, i.e., "to understand the effects of the use of strobilurin fungicides in regard of fertilizer N rates on morphology of winter wheat", two null hypothesis were set.

Firstly this chapter will test the hypothesis that there is no synergistic effect between the use of strobilurin fungicides and fertilizer N rates on plant height and Specific Leaf Area (SLA). Secondly it will test the hypothesis that the use of a strobilurin fungicide in a disease control programme in wheat does not affect plant height and Specific Leaf Area (SLA).

Three field experiments under a factorial design of fungicide programmes and N rates as factors were performed. The fungicide programmes included the use of the triazole, epoxiconazole alone, and in mixture with either kresoxim-methyl or trifloxystrobin, which are strobilurins. A range of N rates were used which varied according to the specific requirements for each field experiment.

5.2 Materials and Methods

The data from Cycle II, Cycle III-B and Cycle III-C were used in this chapter.

5.2.1 Plant Height

Plant height of the aboveground part was measured for 20 plants per each plot in Cycle II and Cycle III-B at all the samplings and in Cycle III-C at pre-harvest. Skeleton analysis of variance for each field experiment is given in Chapter 1.

5.2.2 Specific Leaf Area (SLA)

SLA is defined as the area per unit dry matter weight of green leaf.

5.3 Results

5.3.1 Plant Height

5.3.1.1 Aboveground Plant Height

In Cycle II, there was no interaction in aboveground plant height between factors/treatments. Aboveground plant height was reduced by a mixture of epoxiconazole and trifloxystrobin compared to untreated and other fungicide programmes (Table 5.3.1.1 (a)). There was no interaction in aboveground plant height between fungicide programmes and N rates in Cycle III-B and Cycle III-C. In Cycle III-B, the plots treated with epoxiconazole alone and those treated with a mixture of epoxiconazole and trifloxystrobin showed a shorter aboveground plant height than untreated plots at 255 – 264 days after sowing ($P = 0.004$), at 269 – 288 days after sowing ($P < 0.001$) and at 295 – 302 days after sowing ($P = 0.017$) (Table 5.3.1.1 (b)). Such an effect of fungicides on aboveground plant height was not observed in Cycle III-C at pre-harvest (Table 5.3.1.1 (c)).

Table 5.3.1.1 (a) Aboveground Plant Height (cm) in Cycle II

Fungicide/DAS	*168-172*	*186-193*	*214-219*
untrt	53.6	54.1	53.6
epoxi	53.4	54.1	53.1
epoxi + kreso	54.2	53.9	53.7
epoxi + triflo	50.8	51.7	51.5
P value	< 0.001	= 0.002	= 0.027
L.S.D.	1.4	1.4	1.59
CV %	3.9	3.9	4.5

untrt: untreated; epoxi: epoxiconazole; kreso: kresoxim-methyl; triflo: trifloxystrobin

Table 5.3.1.1 (b) Aboveground Plant Height (cm) in Cycle III-B

Fungicide/DAS	*194-198*	*237-244*	*255-264*	*269-288*	*295-302*	*311-323*
untrt	35.6	66.4	81.9	84.5	84.2	83.5
epoxi	34.5	65.2	79.1	80.5	81.7	81.1
epoxi + triflo	34.1	65.0	79.5	81.1	81.3	80.8
P value	= 0.089	= 0.275	= 0.004	< 0.001	= 0.017	= 0.060
L.S.D.	NS	NS	1.7	1.8	2.1	NS
CV %	4.7	3.3	2.5	2.6	3.0	3.5

untrt: untreated; epoxi: epoxiconazole; triflo: trifloxystrobin

Table 5.3.1.1 (c) Aboveground Plant Height (cm) in Cycle III-C

Fungicide/DAS	*314-317*
untrt	84.6
epoxi	84.0
epoxi + kreso	84.3
epoxi + triflo	84.0
P value	0.585
L.S.D.	NS
CV %	1.6

untrt: untreated; epoxi: epoxiconazole; kreso: kresoxim-methyl; triflo: trifloxystrobin

5.3.1.2 Internode length in Cycle II

There was no interaction in internode length between treatments/factors in Cycle II. The plots treated with a mixture of epoxiconazole and trifloxystrobin showed a shorter internode length between leaf 1 and leaf 2 as well as between leaf 2 and leaf 3 than untreated plots, the plots treated with epoxiconazole alone and those treated with a mixture of epoxiconazole and kresoxim-methyl both at 168 – 172 days after sowing and at 186 – 193 days after sowing (Table 5.3.1.2). An attempt was made to relate internode length with the severity of *Septoria* diseases, but it was not successful (data not shown).

Table 5.3.1.2 Internode length (cm) in Cycle II

Fungicide Programmes	*Internode 1-2**		*Internode 2-3***	
DAS	*168-172*	*186-193*	*168-172*	*186-193*
untrt	17.5	17.50	7.1	7.4
epoxi	17.4	17.49	7.1	7.3
epoxi + kreso	17.5	17.40	7.3	7.3
epoxi + triflo	16.6	16.71	6.3	6.7
P value	< 0.001	< 0.001	= 0.001	= 0.004
L.S.D.	0.5	0.33	0.5	0.4
CV %	4.4	2.9	10.6	9.0

untrt: untreated; epoxi: epoxiconazole; kreso: kresoxim-methyl; triflo: trifloxystrobin

*internode between leaf 1 and leaf 2

**internode between leaf 2 and leaf 3

5.3.2 Specific Leaf Area (SLA)

In Cycle II fungicide programmes did not affect SLA at 184 – 185 days after sowing (Table 5.3.2 (a)). In Cycle III-B at 237 – 244 days after sowing, there was no difference in SLA of leaf 1 and leaf 2 between fungicide programmes, while untreated plots showed a greater SLA of lower green leaf than the plots treated with epoxiconazole alone and those treated with a mixture of epoxiconazole and trifloxystrobin by 7 $cm^2\ g^{-1}$ and 8 $cm^2\ g^{-1}$ respectively (P = 0.019) (Table 5.3.2 (b)). At 255 – 264 days after sowing, untreated plots showed a smaller SLA of lower green leaf than the plots treated with epoxiconazole alone and those treated with a mixture of epoxiconazole and trifloxystrobin by 8 $cm^2\ g^{-1}$ and 12 $cm^2\ g^{-1}$ respectively (P = 0.025) (Table 6.3.2 (b)). At 269 – 288 days after sowing, the plots treated with a mixture of epoxiconazole and trifloxystrobin showed a greater SLA of lower green leaf than untreated plots and the plots treated with epoxiconazole alone by 13 $cm^2\ g^{-1}$ and 9 $cm^2\ g^{-1}$ respectively (P = 0.016) (Table 5.3.2 (b)).

Table 5.3.2 (a) SLA ($cm^2 g^{-1}$) of leaf 1 at 184 – 185 DAS in Cycle II

Fungicide Programmes	*SLA*
epoxi	141.7
epoxi + kreso	141.8
epoxi + triflo	140.5
P value	= 0.552
L.S.D.	NS
CV %	2.7

untrt: untreated; epoxi: epoxiconazole; kreso: kresoxim-methyl; triflo: trifloxystrobin

Table 5.3.2 (b) SLA ($cm^2 g^{-1}$) in Cycle III-B

DAS	*237-244*	*237-244*	*237-244*	*255-264*	*269-288*
Fungicide/Leaf	**Leaf 1**	**Leaf 2**	**LG Leaf***	**LG Leaf***	**LG Leaf***
untrt	213	216	249	203	176
epoxi	211	214	242	211	180
epoxi + triflo	209	212	241	215	189
P value	= 0.382	= 0.354	= 0.019	= 0.025	= 0.016
L.S.D.	NS	NS	6	8	9
CV %	3.2	3.3	3.0	4.8	5.8

untrt: untreated; epoxi: epoxiconazole; triflo: trifloxystrobin

*LG Leaf: lower green leaf

5.4 Discussion

5.4.1 Plant Height and Internode Length

It was shown that plant height was significantly affected by application of fungicides both in Cycle II and Cycle III-B. In Cycle II plant height as well as internode length was reduced following the application of a mixture of epoxiconazole and trifloxystrobin but not following that of a mixture of epoxiconazole and kresoxim-methyl or of epoxiconazole alone. The effect was evident at every sampling conducted during the grain filling period. It is not known why a mixture of epoxiconazole and trifloxystrobin reduced plant height, while the other strobilurin fungicide programme, a mixture of epoxiconazole and kresoxim-methyl did not.

In Cycle III-B, plant height was reduced by the application of a mixture of epoxiconazole and trifloxystrobin as well as that of epoxiconazole alone compared to untreated plots. The effect was absent at 1st (194 – 198 DAS) and 2nd (237 – 244 DAS) sampling following the first fungicide application but became evident after the second fungicide application and continued until the 5th sampling (295 – 302 DAS). It is interesting that in this field experiment, plant height was reduced both by triazole fungicide programme and the combination of triazole and strobilurin fungicide programme. Complex interactions of fungicide compounds with the environment are suspected to exist. It is of interest whether dwarfing effects of fungicides has any physiological consequence such as reduced water consumption and improved water use efficiency as suggested by Sumi and Katayama (2001) for dwarf isogenic lines. Internode length could not be related to the severity of *Septoria* diseases in this study (data not shown), however, shortened statue of the plant following the application of fungicides might have an implication for the development of *Septoria* diseases, as vertical movement of inoculum by rain splash plays a role in inoculum transfer within the canopy of wheat

crops (Royle *et al.*, 1986). Supposing that shortened statue of the plant favours inoculum transfer of *Septoria* diseases, at least, to some extent, it would be difficult to detect such an effect, as the main effect of fungicides is obviously to keep the diseases at as a low level as possible.

5.4.2 Specific Leaf Area (SLA)

The effect of fungicides on specific leaf area (SLA) was even more complicated than plant height. No effect of fungicides on SLA was observed in Cycle II, however, assessment was conducted only at one occasion in this field experiment. In Cycle III-B, the effect of SLA of lower green leaf was observed at 2nd (237 – 244 DAS), 3rd (255 – 264 DAS) and 4th (269 – 288 DAS) sampling. Considering that the second fungicide application took place at 242 days after sowing when the 3 out of 4 blocks of sampling was finished at 2nd sampling, observed effect of fungicides on SLA at this sampling was probably attributable to the first fungicide application conducted at 174 days after sowing. It should be noted that at 2nd sampling, the plots treated with fungicides showed a smaller SLA than untreated plots, while at 3rd sampling they showed a greater SLA than untreated plots. At 4th sampling the plots treated with a mixture of epoxiconazole and trifloxystrobin showed a greater SLA than both untreated plots and those treated with epoxiconazole alone. With cleavers (*Galium aparine* L.), Benton and Cobb (1995) observed a reduction in leaf area as well as an increase in leaf thickness following the application of epoxiconazole. In the present study, unfortunately leaf thickness was not measured. However, it is considered that an increased SLA would suggest a reduction in leaf thickness rather than an increase. Physiological consequence of increased SLA should be evaluated in terms of crop growth and yield.

Chapter 6

Photosynthesis and Estimation of Biomass Accumulation

6.1 Introduction

6.1.1 Measurement and Estimation of Rate of Photosynthesis

Photosynthetic rates cannot be directly measured but calculated from measured values such as, in the case of the chamber method, influx and efflux of CO_2 and H_2O concentration and temperature using a number of theoretically derived equations. Thus, it is a prerequisite to measure these values under a justifiable condition in order to calculate photosynthetic rates. It has been known that photosynthetic rates are highly influenced not only by factors associated with the state of the measured plant but also by many environmental factors such as radiation, temperature, humidity and wind speed. The environment inside the chamber used for photosynthetic measurements is very different from that in the open-air situation where crops are normally grown and therefore, one has to be aware of the discrepancy between estimated and actual photosynthetic rates. Measured photosynthetic rates are very often lower than actual ones and this is attributable to a number of factors such as insufficient mixing of the air inside the chamber leading to unusually high boundary layer resistance and insufficient aperture of stomata due to a large difference between leaf and chamber-air temperature and/or humidity (Horie, 1981).

6.1.2 Plant Factors in Photosynthesis

6.1.2.1 Leaf Age

The ability of a given leaf to photosynthesize reaches maximum at its full expansion followed by a gradual decline accompanied by a breakdown of chloroplasts as well as enzymes (Salisbury and Ross, 1992). Along the aging process, leaves show anatomical change in response to the particular environment where they are placed. When leaves are placed on the top of the canopy receiving full sunlight, they are thicker and may form longer palisade cells compared to shade leaves exposed to less sunlight.

6.1.2.2 Leaf N Concentration

As a substantial fraction of leaf N is associated with the photosynthetic apparatus, it is logical to find a high positive correlation between leaf N concentration and leaf photosynthetic rate (Sinclair and Horie, 1989). Up to 50 % of soluble protein in C_3 leaves is ribulose 1,5-bisphosphate carboxylase, the enzyme that catalyzes the reaction of CO_2 with ribulose 1,5-bisphosphate to yield two molecules of 3-phosphoglycerate (Sinclair and Horie, 1989; Buchanan, 1998). Ishihara *et al.* (1978; 1979a) found larger stomatal aperture and diffusion conductance with leaves of higher N concentration than those of lower N concentration in rice. Ishihara *et al.* (1979b) compared photosynthetic rates of rice measured with two methods, the infrared gas analyzer method which is influenced by stomatal aperture and conductance and the oxygen electrode method which is less affected by these factors, and found that although photosynthetic rates measured with both of the methods showed an increase as leaf N concentration increased, the increase rate was less for the oxygen

electrode method than for the infrared gas analyzer indicating that the difference in the increase rate between the two methods is attributable to the effect of N on stomatal aperture and conductance. It could be deduced from these results that leaf nitrogen concentration affects photosynthetic rate at least partly through stomatal aperture and conductance.

6.1.3 Environmental Factors in Photosynthesis

6.1.3.1 Irradiance

The light response photosynthesis curve has played a central role in photosynthetic research both at leaf and canopy level. This is not surprising considering that photosynthesis is a light-driven redox process (Taiz and Zeiger, 1998). Photon flux density decreases exponentially as it penetrates a plant canopy in a downward direction (Monsi and Saeki, 1953), which has been often applied to modeling canopy photosynthesis. Admitting that radiation is the most important factor to determine CO_2 assimilation of crops, some researchers have raised the concern that consideration of radiation alone does not sufficiently quantify dry matter accumulation by photosynthesis, as photosynthetic rates are influenced by more factors especially microclimate surrounding plant canopy (Horie, 1981; Yabuki, 1990).

6.1.3.2 Temperature

In photosynthesis, dark response is more affected by temperature than photo response, as the dark response involves a number of enzymes to process chemical reactions (Matsuo *et al.*, 1990). Spiertz (1977) carried

out an experiment in a phytotron and measured the rate of apparent photosynthesis of wheat flag leaves under four different temperature regimes ranging from 10 to 25 ^{0}C at three weeks after anthesis. The highest photosynthetic rate was observed under 15 ^{0}C. He suggested that leaves senesced faster under higher temperatures to explain the lower photosynthetic rates observed for the temperature regimes of 20 and 25 ^{0}C. Increased rates of CO_2 evolution from photorespiration under high temperature may cause a reduction in photosynthetic rate as well (Farquhar and Sharkey, 1982).

6.1.3.3 Humidity

It is well known that cumulative dry matter accumulation shows a linear relationship with cumulative transpiration (Loomis and Connor, 1992). Since their movement to land in the course of evolution, plants have been facing the dilemma of losing water while obtaining CO_2 for assimilation by opening stomata. The mechanism of opening and closing stomata is complex, but at least it can be said that a steep vapour gradient between the air and intercellular spaces is one of the factors that induces closing of stomata (Salisbury and Ross, 1992). For reliable measurement of CO_2 exchange rate, it is crucial that the vapour gradient be kept under critical level where stomata start closing.

6.1.3.4 CO_2 concentration

The effects of CO_2 concentration on photosynthetic rates can be understood when the difference in the ability of photosynthesis between C_3 and C_4 plants is considered. What C_4 plants do is to concentrate CO_2 in bundle sheath cells, which efficiently prevents photorespiration from occurring (Taiz and Zeiger, 1998). In

C_3 plants, increasing the ratio of CO_2 to O_2 is known to increase photosynthetic rate by reducing photorespiration (Salisbury and Ross, 1992), which can be observed as one of the consequences of the increase in the air CO_2 concentration due to human activity of burning fossil fuels in large quantities since the time of the industrial revolution.

6.1.4 Crude Estimation of Biomass Accumulation

6.1.4.1 Why Estimation of Biomass Accumulation?

One of the major interests of agronomists is yield, not only the edible part of the crop but also total biomass accumulation during the growing period. As crop production is considered to be a process that aims at conversion, as efficient as possible, of solar energy into biomass, it is important to study how environmental as well as management factors affect, in a quantitative manner, the conversion process. If one understands rather well a given crop production system in a quantitative manner, he/she should be able to integrate a number of factors involved in the system and roughly estimate the output, i.e. biomass accumulation. When it happens that the modeled reality shows a discrepancy from the observed reality, he/she then should be able to analyze what has caused such a discrepancy between the model and the reality to enhance his/her understanding of the studied system.

6.1.4.2 Estimating Biomass Accumulation from Leaf N Status

Many studies have examined the relationship between leaf nitrogen content and light-saturated rate of leaf

CO_2 assimilation and found high positive correlations between the two (Evans, 1983; Evans, 1989; Sinclair and Horie, 1989; Shiraiwa and Sinclair, 1993; Bindraban, 1997; Dreccer, 1999) where asymptotic curves are often suggested to explain the relationship for a number of crops. As CO_2 assimilation rate varies with the level of photon flux density available at each moment, it needs to be theoretically estimated for relatively short period of time, at the same time considering the way light diminishes as it penetrates a given canopy (Monsi and Saeki, 1953) of the crop. Accumulating the estimated CO_2 assimilation rate for each second would give the amount of CO_2 assimilated during the period of accumulation, which can be crudely converted into biomass weight (Penning de Vries *et al.*, 1974). Comparison of the estimated biomass accumulation with the measured increase in crop dry matter weight could provide a tool to enhance the understanding as to how nitrogen status of the crop is reflected into biomass accumulation as well as the extent to which a certain treatment, especially the one that is likely to affect nitrogen status of the crop, could influence the process of biomass accumulation of the crop.

6.1.5 Objectives of Chapter 6

Two objectives were set (Obj. 6-1 and Obj. 6-2) in this chapter, one with respect to the measurement of CO_2 exchange rate (CER) and the other to address the objective (I) described in the section of "*1.2 Aim, Objectives and Approach*" of Chapter 1, i.e., "to quantitatively understand the relationship between N accumulation and biomass accumulation of winter wheat with the aid of systems approach and modeling". After failing to obtain reliable photosynthesis data sets in the previous two years, there was only one

objective of photosynthesis measurement in the third year. That was to obtain a relationship between leaf N concentration and maximum gross photosynthetic rate under saturated irradiance of single leaves under optimum condition for the equipment. The objective of statistically detecting any possible differences in photosynthetic ability of wheat plants treated with different nitrogen and fungicide treatments was abandoned, as many factors such as the state of the equipment, varying field weather conditions, limitations on the number of measurements that can be taken does not seem to allow a collection of the data sets that are capable of fulfilling the objective. Attempts are therefore to be made to test whether differences in biomass accumulation between different N and fungicide treatments could be explained, to any extent, through a relationship between leaf N concentration and CER using modeling approach.

Obj.6-1

To obtain a relationship between leaf N concentration and maximum gross photosynthetic rate under saturated irradiance of single leaves under optimum condition for IRGA

Obj.6-2

To test whether differences in biomass accumulation between different N and fungicide treatments could be explained, to any extent, through a relationship between leaf N concentration and CER using modeling approach.

6.2 Materials and Methods

6.2.1 Preliminary Measurements

Several measurements were taken in order to determine the best method to obtain data sets under various constraints such as the state of equipment and limited time available for measurement as discovered through the previous experiments.

6.2.1.1 Sampling method

From the previous two-year's-attempts of taking photosynthesis measurements in the field, it was found that moving the equipment from one spot to another takes a long time thus reducing the actual measurements. Therefore, the possibility of sampling plants from the field and taking the measurement in a fixed spot was explored. Two methods, air sampling and water sampling were compared. Ten winter wheat tillers were cut at the ground level (air sampling) in the afternoon on 3 May 2002. Five tillers were used to take measurement in the same afternoon and another five were kept with the bottom of their stems in the water overnight prior to measurement. Ten winter wheat tillers were cut in a basin full of water (water sampling) and measurement was taken in the same morning on 4 May 2002.

6.2.1.2 Leaf Area Determination

The Delta T involves up to 10 % error in determination of leaf area and it is not appropriate to use it for determination of a single leaf area. The leaf area used for photosynthesis measurement was calculated as a

rectangle from leaf width and leaf length fitted within the leaf chamber.

6.2.1.3 Irradiance

A sudden exposure of a leaf to photosynthetic photon flux density (PPFD) of as strong as 1000 micro mol m^{-2} s^{-1} may encounter the risk of photoinhibition causing a severe reduction of the rate of photosynthesis (Mohr and Schopfer, 1995). It is necessary to give plants a chance to acclimatize prior to imposing such a high level of PPFD. In this study, it was achieved by exposing leaves initially to relatively a lower level of PPFD followed by a gradual increase.

6.2.1.4 Temperature

There is not much that could be done to control temperature except for choosing early hours in the morning to take measurements. It is known that temperature higher than 30 °C severely reduces photosynthetic rate of wheat plants.

6.2.1.5 CO_2 concentration

As the maximum flow rate of the available equipment (i.e. 450 ml min^{-1}) was not sufficient for the available chamber, CO_2 concentration of the inflow air was set at a higher level than that of ambient air to minimize the degree of CO_2 depletion in the chamber.

6.2.2 Crude Estimation of Biomass Accumulation

Daily solar radiation measured at the nearest weather station (Newport, Shropshire) was used to crudely estimate the amount of solar radiation for the periods of 30 minutes for each day. For each period of 30 minutes, the average solar radiation was calculated proportionately to the sine curve (i.e. f (x) = sin x) (Shiraiwa, P.C.). The proportion of photosynthetically active radiation (PAR) to the solar radiation was assumed to be 50 % for simplicity (Loomis and Connor, 1992: Dreccer, 1999). The estimation method of CO_2 exchange rate followed the literature (Sinclair and Horie, 1989; Sinclair and Shiraiwa, 1993; Bindraban, 1997). Biomass accumulation was estimated for the period of 20 days between the 2nd and the 3rd sampling. Daily LAI was estimated by linearly interpolating the measured LAI at the 2nd and the 3rd samplings. SLN was measured only at the 2nd sampling, therefore, the SLN at the 2nd sampling was used assuming the SLN to be constant during the period of estimation.

Step 1: Estimation of C^*_A from leaf N (Bindraban, 1997)

$$A_{max} = 25.59 + 46.43 \times \log (\text{leaf } N_a)$$

$$C^*_A = A_{max} \times 10^6 \times 10^{-4} \times (1/3600)$$

A_{max} : light-saturated CO_2 assimilation rate (kg CO_2 ha^{-1} h^{-1})

N_a : leaf N on area basis (g N m^{-2})

C^*_A : light-saturated CO_2 assimilation rate (mg CO_2 m^{-2} s^{-1})

Step 2: Estimation of C_A from C^*_A (Sinclair and Horie, 1989: Sinclair and Shiraiwa, 1993)

Step 2-1: Calculation of fraction of intercepted radiation

As measured radiation extinction coefficient was not available in this study, it was set at 0.44 from the literature (Dreccer, 1999) as described in section *3.2.4* in Chapter 3.

$$F = 1 - \exp(-K \times LAI)$$

K: radiation extinction coefficient (-)

F: fraction of intercepted radiation (-)

LAI: leaf area index (-)

Step 2-2: Calculation of LAI_{sun} and LAI_{shade}

$$LAI_{sun} = F/K$$

$$LAI_{shade} = LAI - LAI_{sun}$$

LAI_{sun} : the LAI intercepting the direct-beam radiation (-)

LAI_{shade} : the LAI exposed only to scattered radiation (-)

Step 2-3: Calculation of I_{sun} and I_{shade}

The assumption is made that 20 % of the intercepted radiation is scattered by the sun leaves and spread uniformly over the shade leaves (Sinclair and Horie, 1989).

$$I_{sun} = I_o \times F/LAI_{sun}$$

$$I_{shade} = 0.2 \times I_o \times F/LAI_{sun}$$

I_o : the radiation flux density incident to the canopy (MJ m^{-2} s^{-1})

I_{sun} : the radiation flux density on the leaves intercepting direct bean radiation (MJ m^{-2} s^{-1})

I_{shade} : the radiation flux density on the leaves intercepting only scattered radiation (MJ m^{-2} s^{-1})

Step 2-4: Calculation of CO_2 assimilation

The value of E, light use efficiency at low light was set as 5.0 g CO_2 MJ^{-1} (Ehleringer and Bjorkman, 1977).

$C_A = C^*_A \{1 - \exp(-E \times 10^3 \times I/C^*_A)\}$ (Boote and Jones, 1987 cited in Sinclair and Horie, 1989)

C_A : CO_2 assimilation (mg CO_2 m^{-2} s^{-1})

I: incident radiation flux density (MJ m^{-2} s^{-1})

E: light use efficiency at low light (g CO_2 MJ^{-1})

$$C_{sun} = LAI_{sun} \times C^*_A \{1 - \exp(-E \times I_{sun} / C^*_A)\}$$

$$C_{shade} = LAI_{shade} \times C^*_A \{1 - \exp(-E \times I_{shade} / C^*_A)\}$$

C_{sun} : CO_2 assimilation by LAI_{sun} (mg CO_2 m^{-2} s^{-1})

C_{shade} : CO_2 assimilation by LAI_{shade} (mg CO_2 m^{-2} s^{-1})

Step 3: Estimation of biomass from C_A (Sinclair and Horie, 1989)

CO_2 assimilation was converted into biomass accumulation, hexose accumulation by multiplying by the ratio of molecular weights per carbon (30/44). Biochemical conversion coefficients developed by Penning de Vries (1974) were combined with maintenance respiration as well as biochemical respiration by the method employed by Sinclair and Horie (1989) for rice and maize.

$$B = 0.6 \times 30/44 \times (C_{sun} + C_{shade})$$

B: biomass (mg *biomass* m^{-2} s^{-1})

6.3 Results and Discussion

6.3.1 Preliminary Measurements

Both transpiration and carbon dioxide efflux were hardly observed for the five tillers that were air sampled and the measurement taken in the same afternoon (data not shown). This phenomenon was described by Dreccer (1999) as "airlock and stomatal closure" and therefore she cut plants under water prior to photosynthesis measurement to prevent it. Net photosynthetic rates obtained from water sampled tillers fell in the range of 4.8 and 24.4 micro mol CO_2 m^{-2} s^{-1} under PPFD of 87 to 282 micro mol m^{-2} s^{-1} and considered to be free from limitations of stomatal closure (Fig. 6.3.1 (a)). Air sampled tillers restarted both transpiration and photosynthesis after soaked in the water overnight (Fig. 6.3.1 (a)) although the possibility of reduction in the rates of these two processes possibly caused by the treatment remained. Thus, it was concluded that water sampling was the better method both for quality of the data and convenience.

In Figure 6.3.1 (b), carbon exchange rate (CER) was plotted against PPFD under both natural and artificial irradiance. Under natural irradiance, CER at higher PPFD does not seem to have reached the light saturated level.

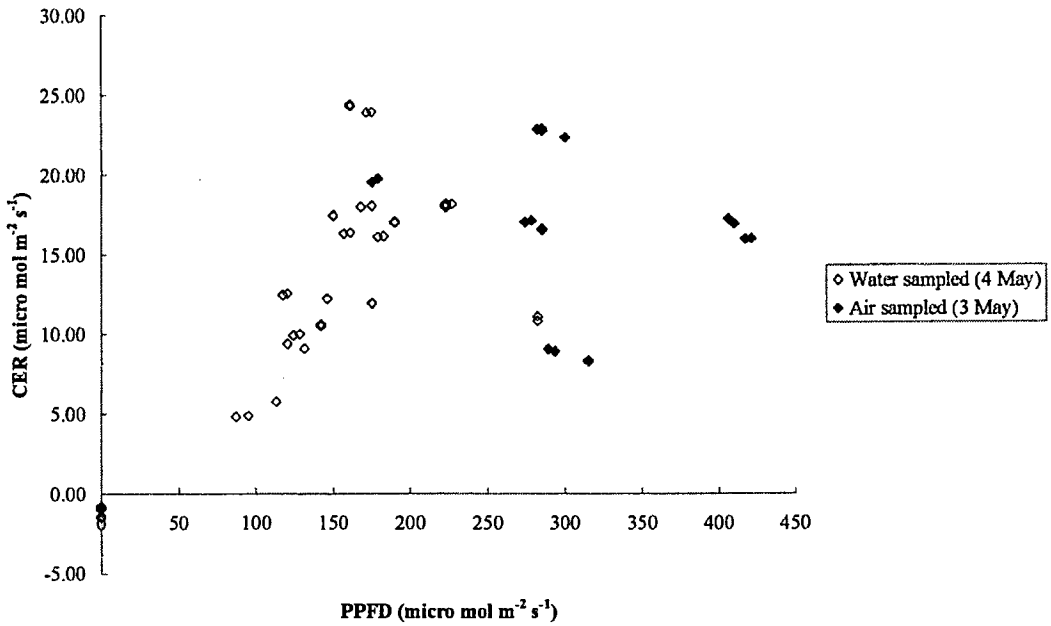


Figure 6.3.1 (a) Photosynthesis light response curve obtained under natural irradiance on a clear sunny day between 8:00 and 10:30 on 4 May 2002 in Cycle III-B

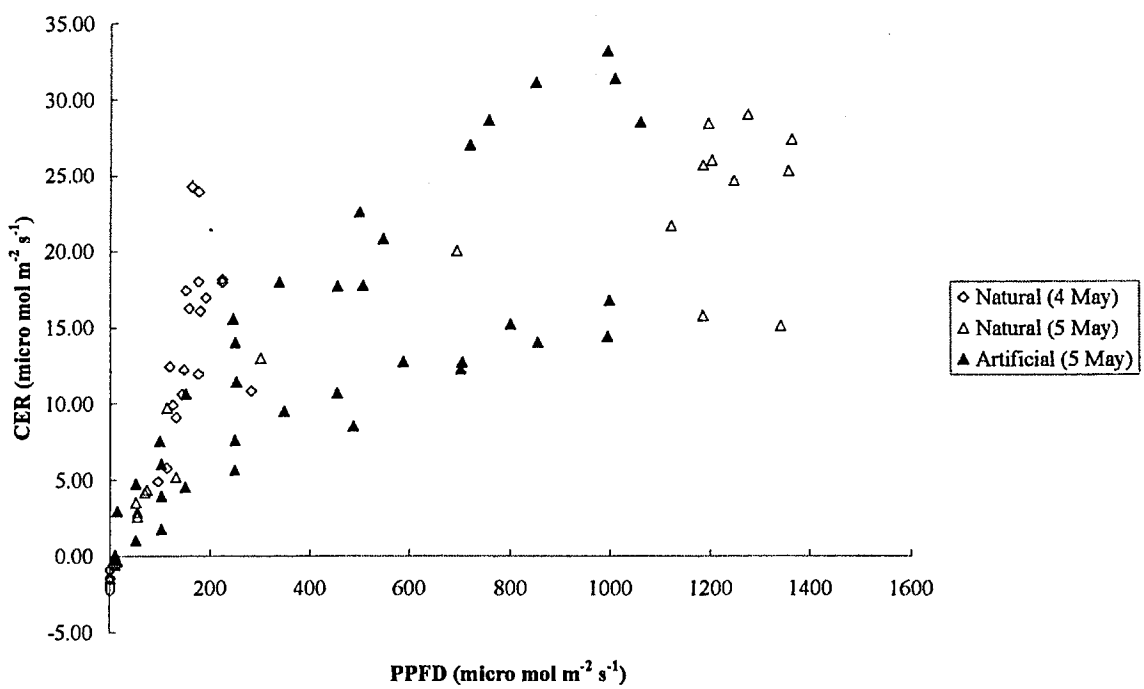


Figure 6.3.1 (b) Photosynthesis light response curve obtained partly under natural irradiance and partly under artificial irradiance on relatively clear sunny days (4 and 5 May 2002) in Cycle III-B

Figure 6.3.1 (c) shows a trend that there is a linear relationship between the rate of transpiration and the rate of carbon exchange rate irrespective of fungicide and N treatments, which was confirmed where a parallel increase in the rate of both transpiration and photosynthesis was observed with the acclimatization of a leaf to the artificial irradiance of 1000 micro mol m^{-2} s^{-1} (Fig. 6.3.1 (d), (e)).

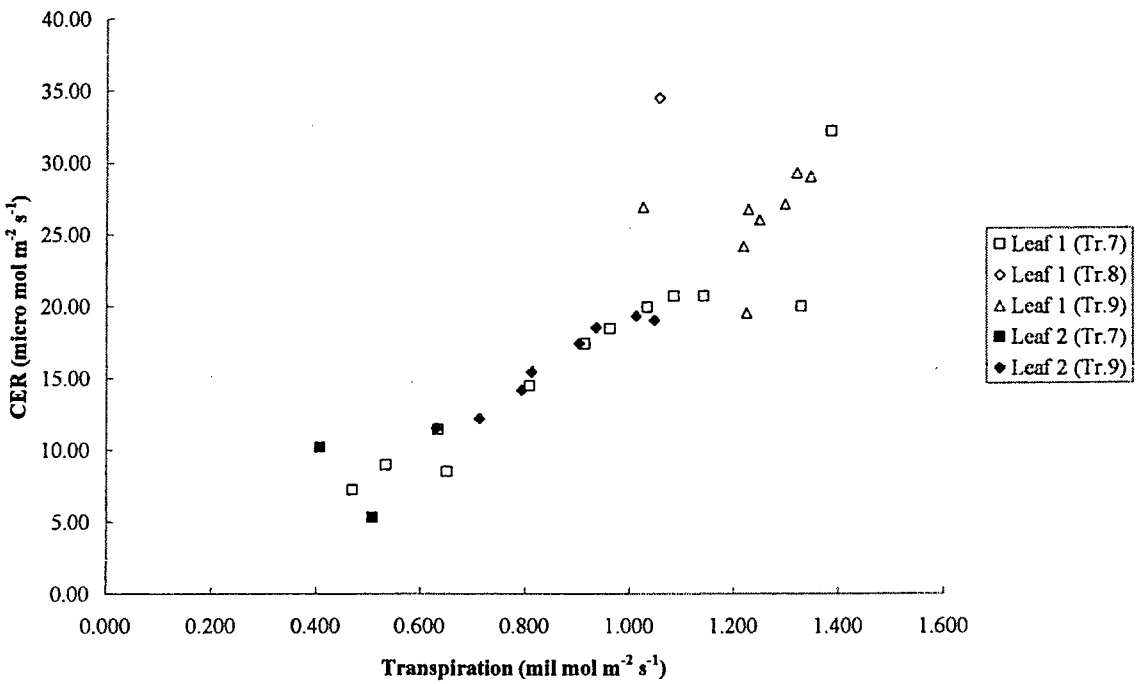


Figure 6.3.1 (c) The relationship between transpiration and CER measured on 19 May 2002 in Cycle III-B

Tr.: Treatment No.

In Figure 6.3.1 (d) and Figure 6.3.1 (e), the process of acclimatization of a leaf to irradiance is shown as to the rate of both transpiration and carbon exchange rate (CER). As to flag leaf, it was almost 30 minutes later that the rate of carbon assimilation became stable (Fig. 6.3.1 (d)). It was shorter for second leaf, however, it still took more than 15 minutes (Fig. 6.3.1 (e)). In both cases, only one leaf was measured and therefore, the time difference is considered to be reflecting the difference in the state of individual leaves

rather than that in the characteristics of flag leaf and second leaf. Adaptation and response of plants to new condition of microenvironment is sometimes a lengthy process and a sufficient acclimatization period needs to be given prior to each measurement. However, time constraints especially imposed from the equipment such as battery life do not always allow plants to carry out sufficient acclimatization or the number of measurements has to be limited.

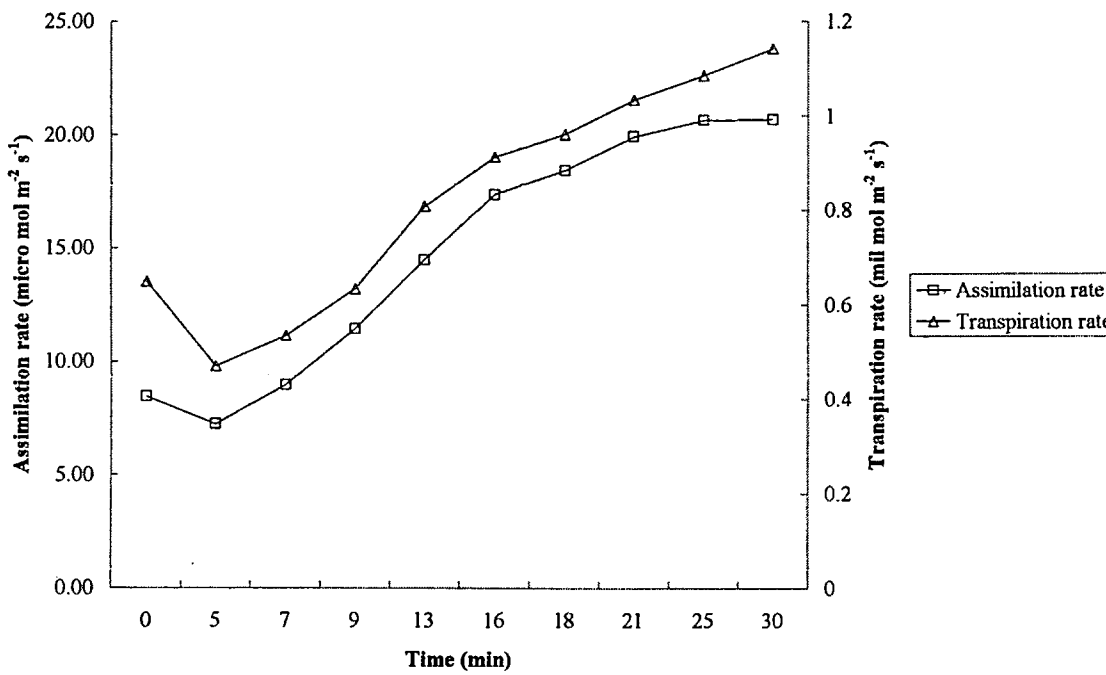


Figure 6.3.1 (d) The time change in assimilation rate and transpiration rate of a flag leaf (Tr. 7) measured at GS 39 on 19 May 2002 in Cycle III-B

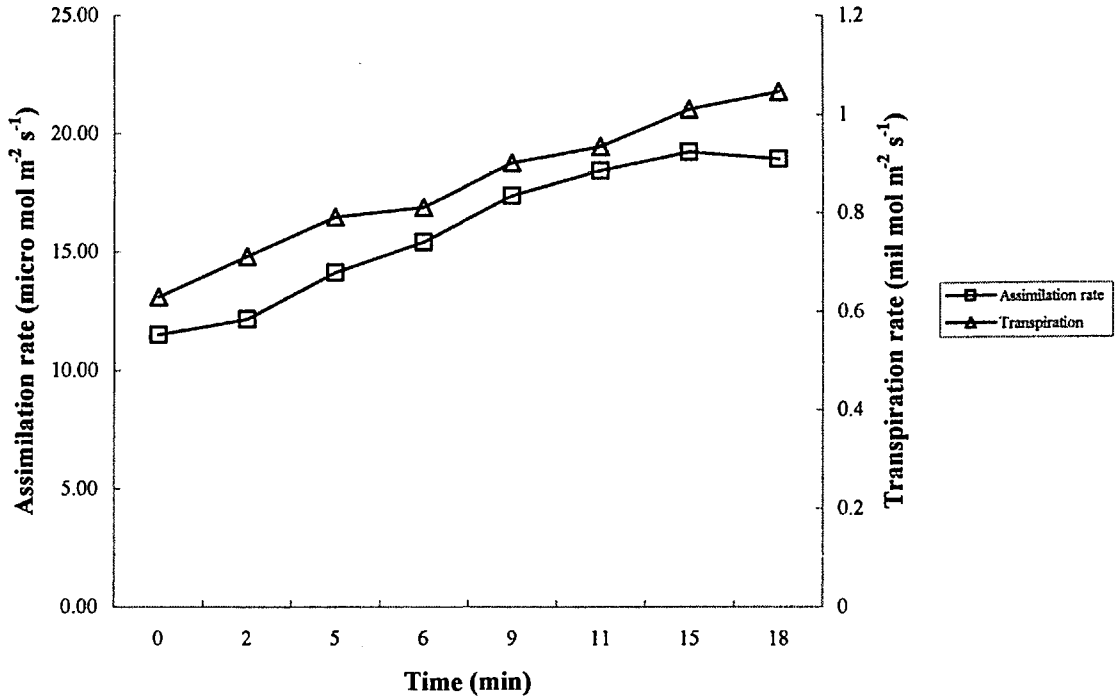


Figure 6.3.1 (e) The time change in assimilation rate and transpiration rate of a leaf 2 (Tr. 9) measured at GS 39 on 19 May 2002 in Cycle III-B

6.3.2 Crude Estimation of Biomass Accumulation in Cycle III-B

6.3.2.1 Leaf N concentration and CO_2 exchange rate (CER)

At GS 39 and GS 59, CER was measured on a number of leaves of the crops from several plots of different treatments in Cycle III-B. Leaf N concentration was plotted against CER (Fig. 6.3.2). Even though the variability of the data sets was too large and the number of data sets was too limited, the relationship between leaf N concentration and CER was compared with that found in the literature (Sinclair and Horie, 1989; Bindraban, 1997; Dreccer, 1999) (Fig. 6.3.2). It was hard to say that the relationship between leaf N concentration and CER obtained from Cycle III-B of this study coincided with any of the literature compared.

However, exclusion of several plots might allow a comparison with Bindraban (1997). For theoretical completeness, the equation of Bindraban (1997) was employed for a crude estimation of biomass accumulation in Cycle III-B.

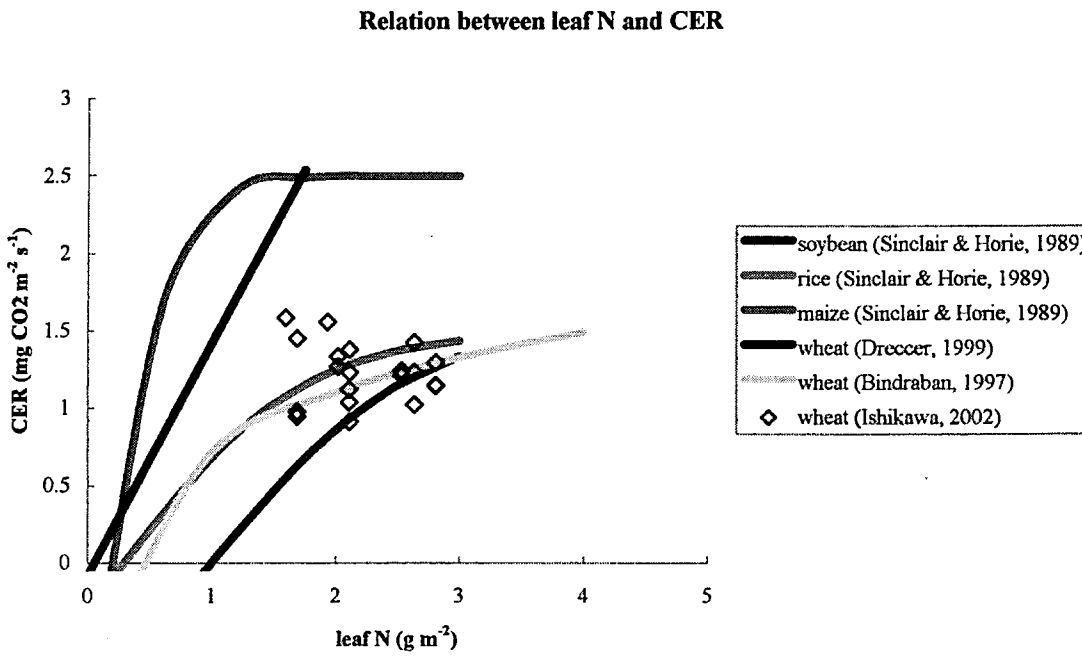


Figure 6.3.2.1 Relationship between leaf N and CER from the literature and the present study

6.3.2.2 Crude Estimation of Biomass Accumulation

Simulating Biomass Accumulation of a Single Day

Daily biomass production was crudely estimated for three days of contrasting irradiance to see the magnitude of difference between the two treatment factors, i.e. fungicide programmes and N rates. The data sets of LAI and SLN used for this calculation are shown in Table 6.3.2.2 (a). The magnitude of the difference in estimated daily biomass accumulation was much smaller for fungicide treatments than for N rates irrespective of the amount of daily solar radiation indicating the difficulty of detecting any difference, even if there is, in dry matter accumulation caused by fungicide programmes compared to that caused by N rates (Fig.

6.3.2.2 (a)).

Table 6.3.2.2 (a) Data sets used for crude estimation of biomass accumulation

Component/Trt	*N-High*	*N-Low*	*untrt*	*epoxi + triflo*
LAI	5.96	2.64	4.72	4.67
SLN (mmol m^{-2})	164	122	144	150

untrt: untreated; epoxi: epoxiconazole; triflo: trifloxystrobin

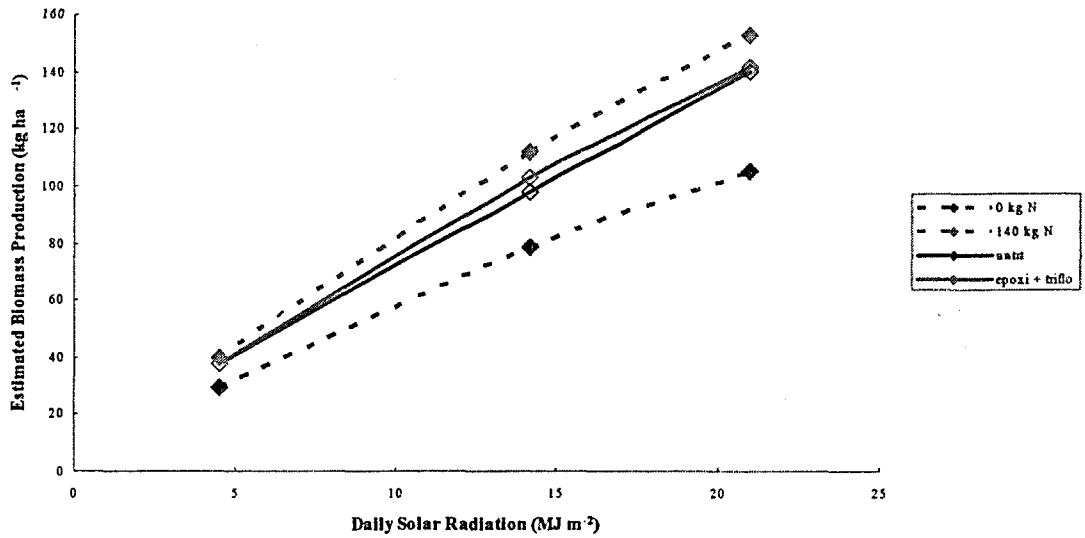


Figure 6.3.2.2 (a) Comparison of crudely estimated daily biomass production for fungicide and N treatments

untrt: untreated; epoxi: epoxiconazole; triflo: trifloxystrobin

kg N: kg N ha^{-1}

Simulating Biomass Accumulation of 20 days

Daily biomass accumulation was calculated for the period of 20 days between the 2nd and the 3rd sampling. The averaged SLN of flag and second leaf (see Chapter 4) and that of second and lower green leaf (see Chapter 4) measured at the 2nd sampling were used for the SLN of LAI_{sun} and LAI_{shade} respectively (Simulation 1; Table 6.3.2.2 (b)). The difference in daily biomass accumulation between fungicide

programmes was hardly recognized until the last part of the period when a reduction in LAI started showing the effects (Fig. 6.3.2.2 (b)). On the other hand, the difference in daily biomass accumulation between N rates was much more evident, even though the difference between the N rate of 100 kg ha^{-1} and that of 140 kg ha^{-1} was not very large (Fig. 6.3.2.2 (c)). The calculated daily biomass accumulation was summed for 20 days and was compared with the measured increase in aboveground dry matter weight during the 2nd and the 3rd sampling, the same period where the simulation was performed (Fig. 6.3.2.2 (d)). Simulated biomass accumulation was found to be severely underestimated by the magnitude ranging from 25 to 32 % (Table 6.3.2.2 (c)). Using the SLN of flag leaf instead of the averaged SLN of flag leaf and second leaf as well as that of second leaf instead of the averaged SLN of second leaf and lower green leaf (Simulation 2; Table 6.3.2.2 (b)) hardly changed the situation (Table 6.3.2.2 (c)). It was supposed that using the relationship between leaf N and CER of the literature might have caused the underestimation. For simplicity, an alternative equation for the relationship between leaf N and CER was derived from the three outliers and the X interception of 0.5 g N m^{-2} (Simulation 3; Table 6.3.2.2 (b)) supposing that outliers might have reflected potential ability of CO_2 assimilation more than other measured rates of CO_2 assimilation (Fig. 6.3.2.2 (e)). Simulated biomass accumulation using the alternative relationship (Eq. 6.3.2.2) between leaf N and CER was increased approximately by 100 kg ha^{-1} in 20 days compared to the original simulation (Simulation 1) using the relationship derived by Bindraban (1997) (Table 6.3.2.2 (c)), however, the magnitude of the increase was not enough to affect the state of underestimation (Table 6.3.2.2 (c)). Manipulation of neither SLN nor the relationship between leaf N and CER managed to solve the problem of underestimation. It was considered that the cause of underestimation should be sought for in other factors, especially parameters. As extinction

coefficient (K) was not obtained in this study, the value of 0.44 was borrowed from the literature (Dreccer, 1999). In order to see the magnitude of the effect of changing the value of extinction coefficient (K), the value of 0.7 was borrowed from Sinclair and Horie (1989) (Table 6.3.2.2 (b); Simulation 4). Changing the value of K resulted in an increased biomass accumulation by 1 to 8 % (Table 6.3.2.2 (c)), which, however, could not give convincing reasoning to explain the discrepancy between measured and simulated biomass accumulation. Finally biomass accumulation in relative value was calculated for each treatment and compared to that of measured biomass accumulation (Fig. 6.3.2.2 (f)). Underestimation of simulated values was observed, even though the degree of underestimation was not as great as that in absolute value. The implication of the observation is the underestimation of the difference in biomass accumulation between treatments. Despite that the measured values of biomass accumulation are susceptible to errors from various sources and that parameters were derived from the literature accepting a number of underlying assumptions, underestimation of as great as 25 to 30 % for all the treatments seems to indicate problems of systematic nature. A possibility is the underestimation of the area of the plant that is capable of carbon assimilation, as LAI used for the simulation was measured only on leaf blade excluding leaf sheath as well as ears. For example, the increase in LAI by 30 % results in increases in biomass accumulation that ranges from 6.4 to 16.7 % and from 2.4 to 11.5 % when K is set at 0.44 and 0.7 respectively (Table 6.3.2.2 (d)). Another cause of underestimation of biomass accumulation might have something to do with the number of days included in the period of simulation in terms of the actual sampling, as only one block out of four could be sampled on a single day. Both of the causes, however, do not seem to explain fully the discrepancy between simulated and measured biomass accumulation in this study. In order to perform simulation of

improved sensitivity, it seems to be necessary to cut down the number of plots to test in order to collect more detailed data sets, which presents a dilemma when performing statistical analysis, for example, ANOVA.

Table 6.3.2.2 (b) Summation of the simulation methods employed for this study

Factor/Method	*Simulation 1*	*Simulation 2*	*Simulation 3*	*Simulation 4*
k	0.44 (Dreccer, 1999)	0.44 (Dreccer, 1999)	0.44 (Dreccer, 1999)	0.7 (Sinclair & Horie, 1989)
SLNsun	(Flag + Second)/2	Flag leaf	(Flag + Second)/2	(Flag + Second)/2
SLNshade	(Second + LG*)/2	Second leaf	(Second + LG*)/2	(Second + LG*)/2
Leaf N and CER	Bindraban (1997)	Bindraban (1997)	Eq. 6.3.2.2	Eq. 6.3.2.2

*lower green leaf

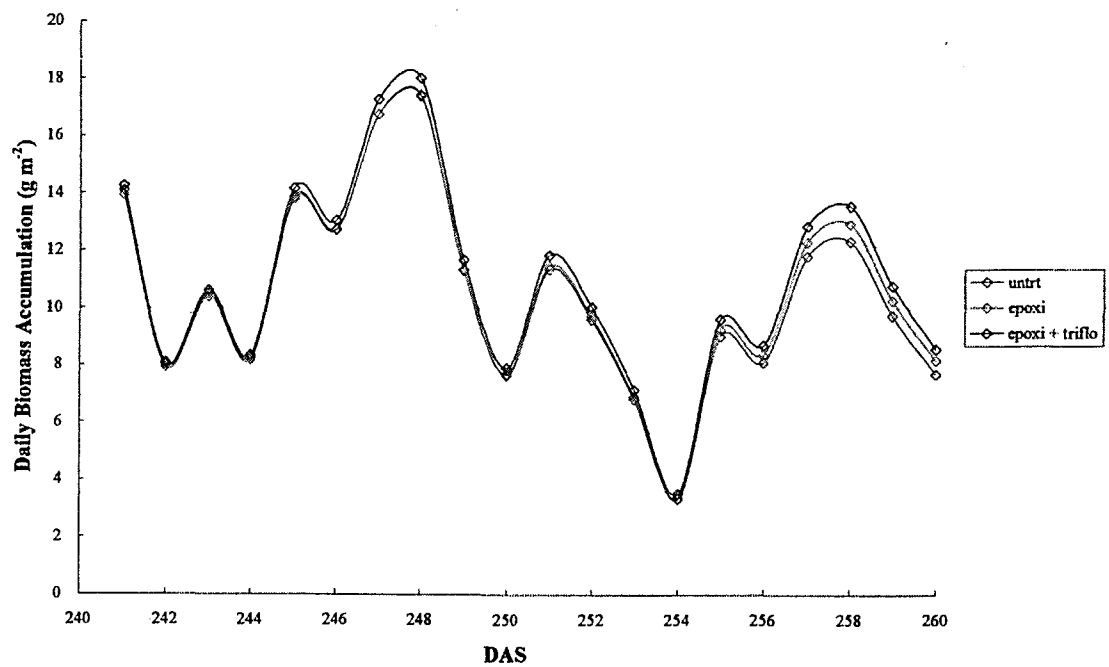


Figure 6.3.2.2 (b) Simulated daily biomass accumulation for fungicide programmes in Cycle III-B

untrt: untreated; epoxi: epoxiconazole; triflo: trifloxystrobin

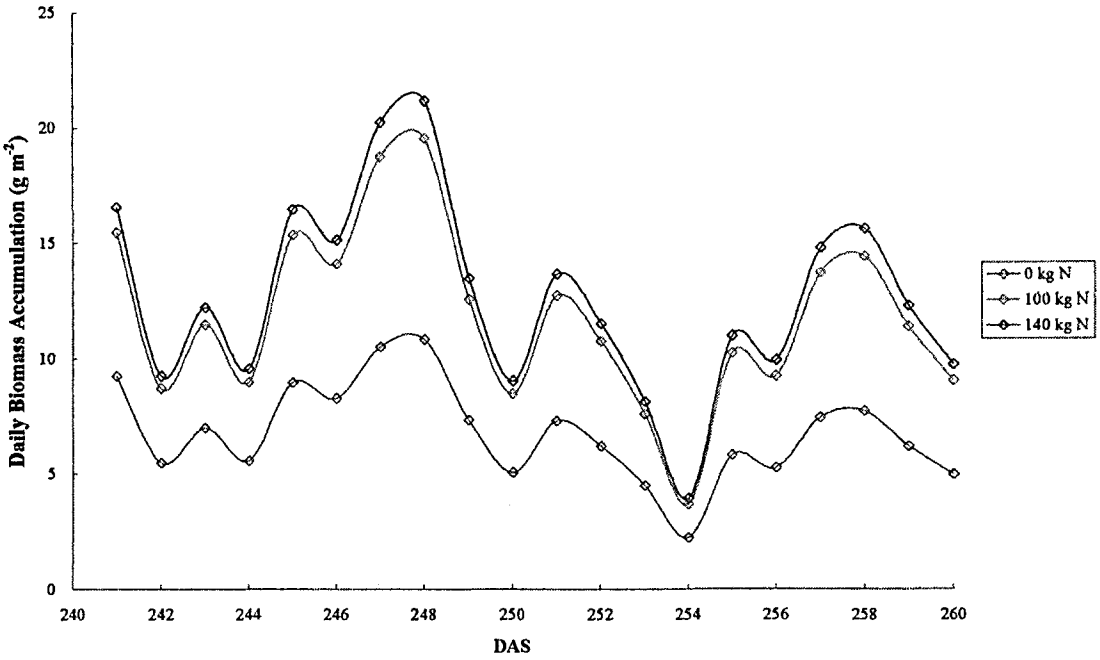


Figure 6.3.2.2 (c) Simulated daily biomass accumulation for N rates in Cycle III-B

kg N: kg N ha^{-1}

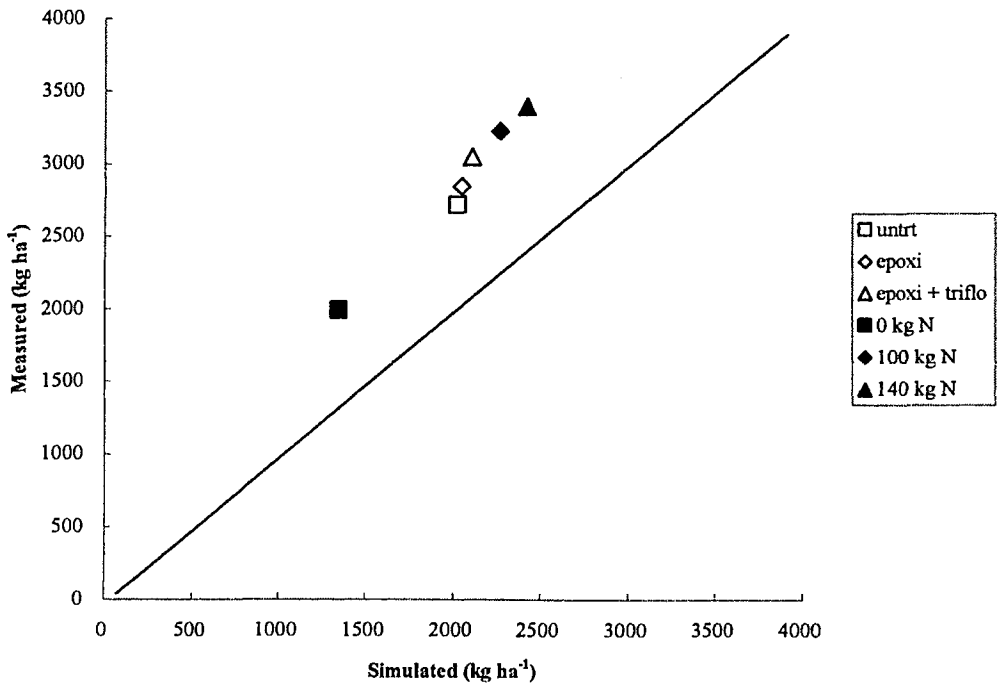


Figure 6.3.2.2 (d) Comparison between simulated and actual biomass accumulation during the period of 20 days between the 2nd sampling and the 3rd sampling in Cycle III-B using the method of Simulation 1

untrt: untreated; epoxi: epoxiconazole; triflo: trifloxystrobin

kg N: kg N ha^{-1}

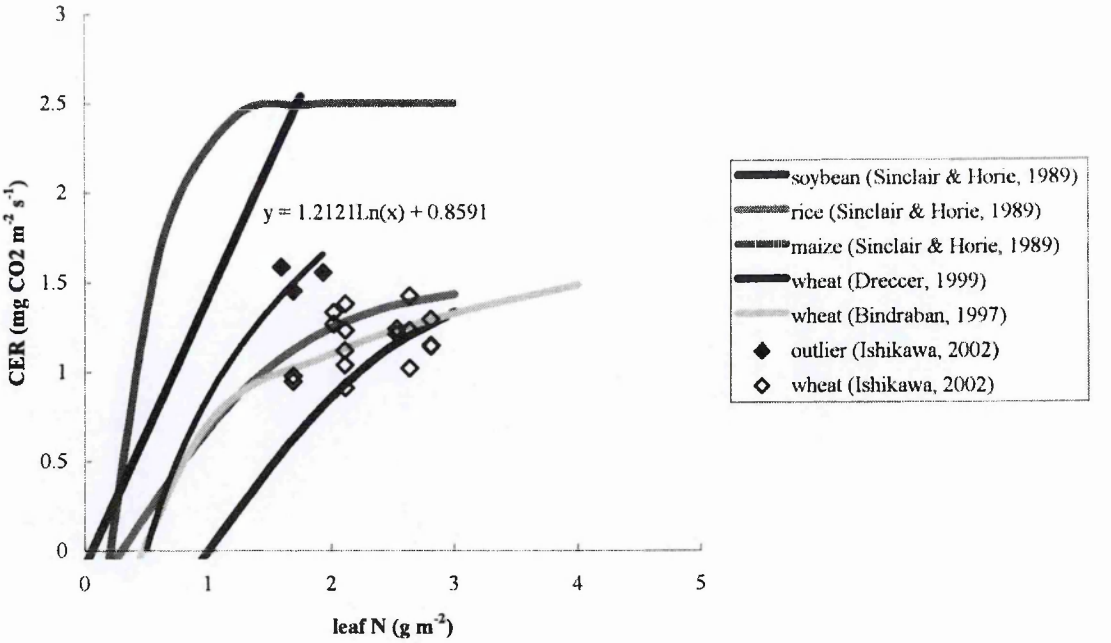


Figure 6.3.2.2 (e) Relationship between leaf N and CER from the literature and the present study estimating a logarithmic equation between leaf N and CER of outliers

CER = 1.2121 LN (leaf N) + 0.8591 (Eq. 6.3.2.2)

Table 6.3.2.2 (c) Simulated biomass accumulation for the 4 simulation methods performed in this study (kg ha^{-1})

Treatment	*Simulation 1*	*Simulation 2*	*Simulation 3*	*Simulation 4*
untrt	2024 (75)	2033 (75)	2100 (77)	2188 (81)
epoxi	2045 (72)	2052 (72)	2126 (75)	2216 (78)
epoxi + triflo	2106 (69)	2113 (69)	2196 (72)	2270 (75)
0 kg N	1344 (68)	1352 (68)	1360 (68)	1502 (76)
100 kg N	2266 (70)	2275 (71)	2364 (73)	2422 (75)
140 kg N	2425 (71)	2432 (72)	2538 (75)	2564 (76)

untrt: untreated; epoxi: epoxiconazole; triflo: trifloxystrobin

kg N: kg N ha^{-1}

*the values between brackets are the relative value to the measured data

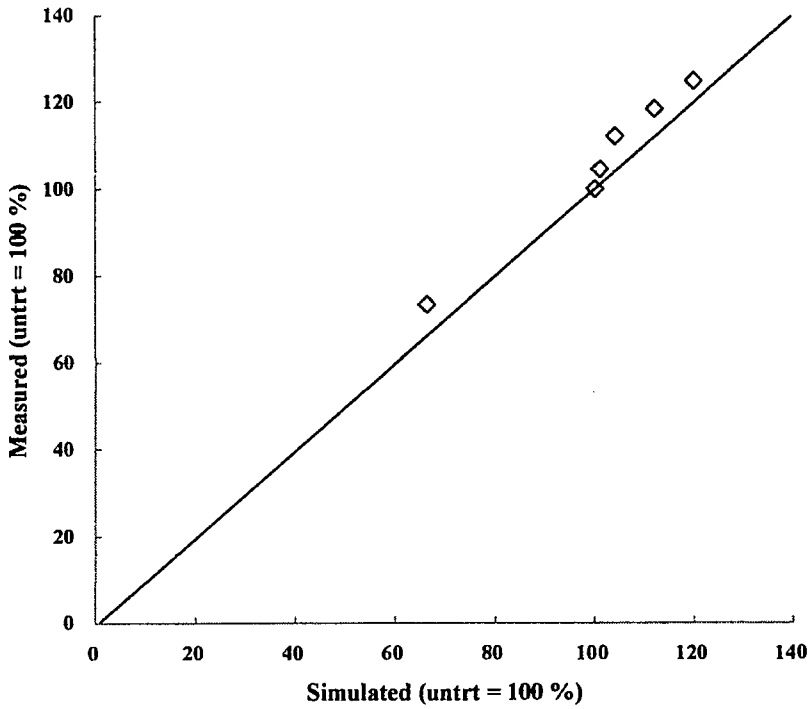


Figure 6.3.2.2 (f) Comparison between simulated and actual biomass accumulation in relative value during the period of 20 days between the 2nd sampling and the 3rd sampling in Cycle III-B using the method of Simulation 1

Table 6.3.2.2 (d) The percentage of increase in simulated biomass accumulation in response to the 30 % increase in LAI for two contrasting *K* values and for three contrasting days of daily solar radiation (%)

K	*Treatment*	*4.5 MJ (254)*	*14.2 MJ (252)*	*28.9 MJ (248)*
0.44	*untrt*	10.9	10.4	9.4
	epoxi	10.5	10.2	9.5
	epoxi + triflo	9.7	9.4	8.8
	0 kg N	16.7	16.4	15.6
	100 kg N	8.8	8.5	7.8
	140 kg N	7.4	7.1	6.4
0.7	*untrt*	5.6	5.2	4.5
	epoxi	5.2	5.0	4.6
	epoxi + triflo	4.6	4.4	4.0
	0 kg N	11.5	11.2	10.3
	100 kg N	3.9	3.7	3.3
	140 kg N	2.8	2.7	2.4

untrt: untreated; epoxi: epoxiconazole; triflo: trifloxystrobin; kg N: kg N ha^{-1}

The number between the brackets is days after sowing (DAS)

Chapter 7

General Discussion

7.1 Interactive Effects of Fungicide Programmes and N rates

7.1.1 Green Leaf Area, Dry Matter Accumulation and Yield

In the present study, no interaction was observed between fungicide programmes and N rates with respect to the severity of *Septoria* diseases, senesced leaf area, LAI and GLAD. These are the traits related to the size and the persistence of green leaf. Therefore, application of fungicides did not appear to affect, in any synergistic manner, the way the crops utilize the applied N in forming and maintaining green leaf. Consequently PAR interception was not affected either in any synergistic manner by fungicide programmes and N rates.

As to DM accumulation, a few synergistic effects were observed between the two treatments. For example in Cycle II, the plots treated with a combination of triazole and strobilurin fungicides, a mixture of epoxiconazole and kresoxim-methyl, responded to a greater extent to the N rate of 130 kg ha^{-1} compared to that of 90 kg ha^{-1} showing an increased aboveground DMW observed immediately after anthesis, while such an effect was not found with the plots treated with other fungicide programmes. A similar observation was made with DMW of rachis and chaff at approximately three weeks after anthesis. There was, however, no evidence that these observed effects of this particular fungicide programme had any beneficial impact on

final yield, as no interaction in DM accumulation was observed between fungicide programmes and N rates at pre-harvest in Cycle II.

The story was slightly different in Cycle III-B where a few interactions were observed with respect to DM accumulation at more advanced stage of grain fill. At approximately six weeks after anthesis, a greater response of aboveground DMW was observed with the N application rate of 140 kg ha^{-1} for the plots treated with a combination of triazole and strobilurin fungicide, a mixture of epoxiconazole and trifloxystrobin this time, than untreated plots and those treated with triazole alone. The implication is that the application of this particular fungicide programme might have improved the way the crops utilized N in carbon assimilation. Looking at it in more detail, similar interactions were found with DMW of spike, flag leaf and second leaf, which consequently resulted in the interaction in aboveground DMW mentioned above. The interesting point to make here is that the synergistic effect of the fungicide programme of triazole and strobilurin with respect to the N rate of 140 kg ha^{-1} was observed on DMW of flag leaf and second leaf. Such an effect was absent in LAI of both of the leaf layers, although the LAI of the plots treated with the combination of triazole and strobilurin appeared to have responded, at least in a numerical sense, to the N rate of 140 kg ha^{-1} to a greater extent than the plots treated with triazole alone ($P = 0.065$) (see Appendix 6).

From the inconsistency of the results between DMW and leaf area of flag leaf, it is tempting to speculate existence of some possible effects of this particular fungicide programme on the ratio of DMW to leaf area of flag leaf, Specific Leaf Area (SLA) in other words. It has to be noted, however, that leaf area of flag leaf

was measured on the part of the leaf that was green at the time of assessment excluding the senesced part, while DM of flag leaf is referred to the total weight including both green area and senesced area. The measurement of SLA made on lower green leaf at approximately three weeks after anthesis showed a significant difference between the plots treated with the combination of triazole and strobilurin and those treated with triazole alone. SLA was greater for the plots treated with the combination of triazole and strobilurin by 9 cm^2 g^{-1}, which might be indicating slightly more efficient utilization of DM by the crops treated with the combination of triazole and strobilurin in maintaining green leaf area. Unfortunately the observations of SLA from a single-year-experiment allow us only to speculate and a further study would be needed to test the hypothesis. Microscopic assessment of leaf structure might be of some use, considering that Benton and Cobb (1995) observed a morphological change in cleavers (*Galium aparine* L.) following the application of epoxiconazole. .

The synergistic effect of the combination of triazole and strobilurin with the N rate of 140 kg ha^{-1} on DMW of spike, flag leaf and second leaf observed in Cycle III-B, however, did not seem to have contributed to the yield in any different manner from the plots treated with the N rate of 100 kg ha^{-1}, while the plots treated with triazole alone showed a significant increase in yield in response to the N rate of 140 kg ha^{-1} compared to that of 100 kg ha^{-1}. Supposing that the increased aboveground DM of the plots treated with the combination of triazole and strobilurin was not fully utilized in forming the yield, there might be some possibility of sink limitation as observed by Ruske *et al.* (2001) with Hereward, the same variety used in this field experiment.

As to yield components, neither ear number per area (ENA) nor grain number per area (GNA) nor TGW nor HI was significantly affected by fungicide programmes and N rates in interactive manner. There was no evidence that the rate of increase in grain DMW, on single grain basis, was interactively affected by fungicide programmes and N rates.

7.1.2 Nitrogen Accumulation

In any of the field experiments in this study, there was no evidence from aboveground N content measured at pre-harvest that aboveground N uptake was affected, in any interactive manner, by fungicide programmes and N rates, however, in Cycle III-B, grain N content measured at harvest indicated a significantly greater N accumulation in grains when the N rate of 140 kg ha^{-1} was applied compared to that of 100 kg ha^{-1} for the plots treated with fungicides, both triazole alone and the combination of triazole and strobilurin, while such an increase in grain N content caused by the increased rate of N application was absent for untreated plots. It is natural to suspect possible interactive effects of fungicide programmes and N rates on N Harvest Index (NHI), considering that only grain N content was increased as the consequence of the interaction between fungicide programmes and N rates, while aboveground N content was not. However, it was not the case in Cycle III-B. It was in Cycle II where complex interactions in NHI between fungicide programmes and N rates were observed at pre-harvest. For the plots treated with the N rate of 90 kg ha^{-1}, there was a significant difference between the plots treated with the two fungicide programmes of the combination of triazole and strobilurin, with the plots treated a mixture of epoxiconazole and kresoxim-methyl showing a greater NHI than those treated with a mixture of epoxiconazole and trifloxystrobin. For the plots treated

with the N rate of 130 kg ha^{-1}, on the other hand, NHI was significantly greater for the plots treated with a mixture of epoxiconazole and trifloxystrobin than untreated plots. It does not seem to be possible to attribute the cause of the interactions to a single factor.

In Cycle II, interactions between fungicide programmes and N rates were observed both in stem N content and leaf N content immediately after anthesis. The tendency was that, with the N application rate of 130 kg ha^{-1}, both stem N content and leaf N content were relatively increased for the plots treated with a mixture of epoxiconazole and kresoxim-methyl compared to untreated plots and those treated with either epoxiconazole alone or a mixture of epoxiconazole and trifloxystrobin. With the N application rate of 90 kg ha^{-1}, neither stem content nor leaf content showed significant differences between fungicide programmes. Despite that Cycle II could not be claimed to be a field experiment conducted on a 'standard crop', it is interesting that the difference in N application rate of as small as 40 kg ha^{-1} caused such a significant change in the way the crop responded to different fungicide programmes. It was not possible to find out whether the increased N content in vegetative organs observed at anthesis as the consequence of the interactive effects of fungicide programmes and N rates contributed to the NHI at pre-harvest. Neither grain N concentration nor vegetative grain N concentration at pre-harvest/harvest was significantly influenced, in any interactive manner, by fungicide programmes and N rates in any of the field experiments conducted in this study. However, when treated with the N rate of 90 kg ha^{-1} and calculated at aboveground plant basis, the plots treated with a mixture of epoxiconazole and kresoxim-methyl showed a slightly but significantly greater N concentration than the plots treated with either epoxiconazole alone or a mixture of epoxiconazole and

trifloxystrobin indicating less DM production at the unit investment of N, in other words, less N use efficiency (NUE) of the plots treated with a mixture of epoxiconazole and kresoxim-methyl. There was no difference in aboveground plant N concentration between fungicide programmes for the plots treated with the N rate of 130 kg ha^{-1}. Although the rate of increase in grain N content was not affected in any interactive manner between fungicide programmes and N rates on single basis, looking at it on unit of land basis gave a different result. The rate of increase in grain N content was relatively enhanced by the application of the N rate of 100 kg ha^{-1} than untreated for the plots treated with fungicides. Between the two fungicide programmes compared, i.e. triazole alone and a combination of triazole and strobilurin, it was only the plots treated with a mixture of epoxiconazole and trifloxystrobin that the rate of increase in grain N content was significantly increased by an increase in the rate of N fertilizer by 40 kg ha^{-1} from the rate of 100 kg ha^{-1} to 140 kg ha^{-1} thus indicating a positive consequence of applying higher N rates to the crops treated with this fungicide programme with respect to grain N accumulation.

7.1.3 Concluding Remarks of 7.1

Although complex interactions were found between fungicide programmes and N rates with respect to the traits measured in this study related to DM accumulation and N accumulation, the general tendency was that some positive synergistic effects were brought about by applying a higher rate of N where strobilurin-based fungicides were applied. There were interactions observed even in Cycle II where there was hardly any difference in performance of the crops treated with triazole alone and that of those treated with combinations of triazole and strobilurin. It can be speculated that physiological effects of fungicides may have been

present even though they were not detected as statistically significant difference in the performance of the crops such as yield and grain quality. Different components of production would determine whether the crops would benefit from management practices such as fungicides, whether strobilurin-containing or not. It is therefore, important to be aware of the characteristics of the fungicides used in relation to diseases, crops and environment.

7.2 Effects of Fungicide Programmes

7.2.1 Fungicide Programmes in Application

As strobilurin fungicides are mainly protectant in action (Anon., 2000b) even though newer products show better performance with respect to curative activity compared to older ones (Anon., 2002c), it has been often recommended that strobilurin fungicides be used in mixture with a triazole which has curative activity especially against *Septoria* diseases (Anon., 2002c). The practice of mixing different compounds also helps in limiting or delaying the build up of resistance of key diseases against strobilurin fungicides. In the UK, however, resistant strains of powdery mildew (Anon., 2000b) and *Septoria tritici* (Anon. 2003b) have been already detected in 1999 and in 2002 respectively. Application of fungicide programmes at the right growth stages is important, however, whether fungicides could be applied at the right timings very much depends on weather conditions particularly wind and rain. In the third year of this study, the second fungicide application aimed at GS 39 was delayed to GS 49-52 and GS 59 for Cycle III-B and Cycle III-C respectively

due to windy conditions that prevailed. However, the delay in fungicide application could not explain why the severity of *Septoria* diseases observed on untreated plots of Cycle III-C was particularly high.

7.2.2 *Septoria* diseases

Assessment of the severity of *Septoria* diseases and of the senesced leaf area both confirmed that there was a meaning of applying fungicides in controlling *Septoria* diseases in years of "usual" weather conditions. Among fungicide programmes, a better performance of the crops was often observed when treated with one of the combinations of triazole and strobilurin tested in this study, i.e. a mixture of epoxiconazole and trifloxystrobin, in maintaining green leaf area for a longer period than those treated with epoxiconazole alone, which has been documented in other studies with other strobilurin-based programmes (Jones, 1998). Such a beneficial effect of applying a mixture of triazole and strobilurin over triazole alone was, however, absent or negligible in the year of abnormal weather conditions as was shown in Cycle II of this study indicating no or very little benefit of applying fungicides to a 'non-standard crop'.

7.2.3 Green Leaf Area, Dry Matter Accumulation and Yield

The difference in the persistence of green leaf area started being observed between untreated plots and those treated with fungicide programmes after anthesis. The difference between the plots treated with triazole alone and those treated with a mixture of epoxiconazole and trifloxystrobin became evident during the later phase of grain fill. The difference between the two fungicide programmes was more evident for the lower leaf layers. Even though it is known that most of the assimilates are derived from carbon assimilation in the

flag leaf during grain fill (Evans *et al.*, 1975), the maintenance of lower leaf layers might be of advantage if, for example, it contributes to an extra uptake of N during grain fill. The advantage of delaying leaf senescence of lower part of the canopy was shown by Ottman and Welch (1988) with respect to DM and N accumulations.

There was no evidence that fungicide programmes are likely to significantly affect aboveground DM accumulation compared to untreated plots except when an exceptionally high level of diseases was observed for untreated plots, but the DMW of some plant parts such as green leaf tended to become greater when treated with fungicide programmes, especially a combination of triazole and strobilurin (i.e. a mixture of epoxiconazole and trifloxystrobin) compared to untreated plots and those treated with triazole alone.

As to yield components, the effects of fungicides appeared to have been limited to TGW (King, 2002). In most cases, yield is strongly determined by grain number per area (Spiertz and Ellen, 1978; Ellen and Spiertz, 1980; Fischer, 1985; Abbate *et al.*, 1995; Bindraban, 1997) rather than TGW unless an extreme reduction in TGW occurs, for example, due to failed crop protection. The magnitude of yield response that fungicides could influence, therefore, seems to be rather limited.

7.2.4 Nitrogen Accumulation

Premature senescence caused by *Septoria* diseases appeared to have trapped N in the senesced leaves as reported by Leitch and Jenkins (1995) therefore reducing NHI. Similarly the difference in NHI observed in

Cycle III-C between fungicide programmes, a mixture of epoxiconazole and trifloxystrobin and that of epoxiconazole and kresoxim-methyl occurred, because more N was kept in the vegetative parts at pre-harvest for the latter than the former. Improved efficiency in N translocation most likely due to prolonged green leaf area duration seems to be the key not to dilute grain protein concentration. Unfortunately it was only the 'non-standard crop' in Cycle II where LAI was measured for the plots treated with a mixture of epoxiconazole and kresoxim-methyl. It was therefore not known in this study whether the application of a mixture of epoxiconazole and kresoxim-methyl was followed by an increased GLAD in Cycle I and Cycle III.

Except for a few interactions with N rates, the overall performance of the plots treated with a mixture of epoxiconazole and kresoxim-methyl was no better than that of those treated with conventional fungicide, epoxiconazole alone. When grain N concentration was considered, the performance of the plots treated with a mixture of epoxiconazole and kresoxim-methyl was even worse than that of those treated with epoxiconazole alone. From this study therefore, there was no evidence that adding kresoxim-methyl to epoxiconazole would bring any advantage to the crop compared to the application of epoxiconazole alone, while there would probably be some benefits obtained by adding trifloxystrobin to epoxiconazole. A greater grain N content observed with the plots treated with a mixture of epoxiconazole and trifloxystrobin compared to those treated with either epoxiconazole alone or a mixture of epoxiconazole and kresoxim-methyl and no significant difference in aboveground N content between fungicide programmes indicated an improved efficiency in N translocation from vegetative organs to grains, which was at least partly confirmed by the

increased NHI for the plots treated with a mixture of epoxiconazole and trifloxystrobin. The part which could not be explained by the increased NHI probably would have been attributable to both increased N uptake and translocation to a degree which was too small to be statistically detectable.

7.2.5 Concluding Remarks of 7.2

Lack of a satisfactory method for quantifying the severity of *Septoria* diseases made it very difficult to distinguish the effects of fungicide programmes on *Septoria* diseases from those on the physiology of the plants. For winter wheat, least benefit was found by Bayles (1999) for low yielding varieties with good resistance to disease, particularly to *Septoria tritici*. As an increased yield observed following applications of strobilurin-based fungicide programmes compared to conventional fungicides is often accompanied by a relatively better control over *Septoria* diseases of the former compared to the latter, the distinction between the two types of effects seems to remain a problem unless complete disease protection is achieved by both of the fungicides, which is not likely to happen in the field. However, clear distinction, even conceptually, of the effects of fungicides on diseases from those on crop physiology is very difficult, considering, for example, that *Septoria* diseases caused a greater amount of N to remain in senesced leaf as observed in this study as well as by others (Leitch and Jenkins, 1995). If physiological processes of a given crop are disturbed by diseases, some physiological consequences on the crop would be naturally expected following the application of fungicides that are applied to control the diseases.

Although much attention has been paid to the difference in performance of the crops treated with

strobilurin-based fungicide programmes compared to those treated with conventional fungicide programmes, little has been known how different chemistry of the same group of fungicides would affect wheat crops. Complex nature of interactions between chemical compounds when applied in a mixture, as is often the case in application to field crops, is easily expected let alone further interactions with the crop and environment in application in the field. By carrying out field experiments at different sites and in different years, at least a trend could be known as to how a given fungicide compound is likely to function on a given crop variety under given environmental conditions.

7.3 Effects of N rates

7.3.1 *Septoria* diseases

Although the severity of foliar diseases is very often regarded to simply either increase or decrease with an increase in N status of host plants, the results of the field experiments conducted in this study suggested a possibility that there might be an optimum N status of host plants for *Septoria* diseases to develop, implying more complex mechanism of the N to affect the severity of *Septoria* diseases. Assessments of *Septoria* diseases found in the literature show the trends of either an increase (Shipton, 1971; Prew *et al.*, 1983; Leitch and Jenkins, 1995) or a decrease (Johnston *et al.*, 1979; Broscious *et al.*, 1985; Arabi *et al.*, 2002) in severity of *Septoria* diseases with increasing N rates, however, a graph showing the relationship between leaf N status and the severity of *Septoria tritici* given by Leitch and Jenkins (1995) appears to have support the idea of

possible existence of optimum host N status for the disease, although they did not see the relationship in their data. As N rates greatly influence the canopy architecture of crops, the possibility that the rates of N application affect the severity of *Septoria* diseases via their influence on the canopy architecture could not be excluded, considering that the process of inoculum transfer of *Septoria* diseases takes place with a great help of rain splash in the crop canopy (Royle *et al.*, 1986). Unfortunately from this study the data could not be obtained that were sufficient to test the hypothesis. However, the observation that the severity of *Septoria* diseases was significantly greater for the plots drilled with the sowing rate of 400 m^{-2} than that of 100 m^{-2} which was made on the field experiment where the effect of seed rates was tested on the severity of *Septoria* diseases, appears to be caused by a dense canopy created by the higher sowing rate.

7.3.2 Green Leaf Area, Dry Matter Accumulation and Yield

N rates played a crucial role in determining the size of leaves of a canopy measured as LAI irrespective of sites and years of different field experiments of this study. The maintenance of greenness was greatly affected by N rates as well, as the observation was made that the greater the N rate, the greater the GLAD. Contrary to traits related to leaf area and its maintenance, aboveground DMW was hardly affected by the difference in N rates as great as 40 kg ha^{-1} to 100 kg ha^{-1} except for the plots that received no N as control. Grain yield was, however, significantly increased by higher N rates. Some of the yield components were found to be under the influence of N rates. For example, ear number per area tended to be increased by higher N rates, even though the difference was not always significant. N was applied at the onset of stem elongation and therefore, was considered to have influenced in the determination process of fertile and

non-fertile tillers. The number of spikelets per ear could be very much influenced by N, but the period of influence is limited to at and close to the double ridge stage (Langer and Liew, 1973) which occurs, in many cases, before GS 30/31, the first N application in this study. It is, therefore, the number of florets per spikelet which N rates would have made the major impact on. GNA was significantly affected by N rates most of the time except for the two N rates (i.e. 100 kg ha^{-1} and 140 kg ha^{-1}) of Cycle III-B. As to TGW, there was a tendency that the greater the N rates, the smaller the TGW, observations similar to which are found in the literature (McCabe and Gallagher, 1993).

7.3.3 Nitrogen Accumulation

The flag leaf is not the only leaf that is benefiting from fungicides. Maintenance of lower leaves might have an implication that the extra N could be kept in the plant for the later demand by grains. More N might be taken up as the consequence of maintaining lower leaves, as assimilates produced by lower leaves are known to be transported to roots where energy is needed for the process of N uptake. The data from Cycle I suggested that N uptake continued during grain filling period for the plots treated with the highest N rate (i.e. 140 kg ha^{-1}), while for the plots treated with the less N rates (i.e. 0 kg ha^{-1} and 100 kg ha^{-1}) appeared to have stopped N uptake earlier. Considering that the difference in N rates was created as early as GS 30/31 and GS 32 and that N was applied as ammonium nitrate, an inorganic form, it is difficult to suppose that the applied N itself directly affected the way the crop took up N during grain filling. It is more likely that the applied N influenced either the mineralization process of organic N in the soil or the physiology of either crop shoots and roots, or both.

In this study, the highest N rates were set following RB209 (Anon, 2000a) and the rest of the N rates were set lower than those. It was therefore natural that many of the plots in this study did not achieve the required protein concentration for bread-making wheat, however, the fact that a considerable number of the plots treated with the highest N rates did not meet the requirement needs to be carefully studied. The two experiments conducted at the same site in the same year i.e. Cycle III-B and Cycle III-C indicated that the N rate of 140 kg ha^{-1} (Cycle III-B) was not enough to achieve the requirement, while that of 200 kg ha^{-1} (Cycle III-C) might have been excessive and not desirable from the environmental point of view. It is, however, probably too early to draw a conclusion that the N rate should have been set at between the rate of 140 kg ha^{-1} and 200 kg ha^{-1}, as in Cycle III-C, half of the plots treated with the N rate of 100 kg ha^{-1}, the rate smaller than that of 140 kg ha^{-1}, achieved the requirement. Even though both of the experiments were conducted at the same site, there might have been differences, no matter how slight, in soil type, microclimate and the amount of N contained in the soil. It is striking that such differences were large enough to cause a crucial difference in grain N concentration between the two experiments.

7.3.4 Concluding Remarks of 7.3

As a consequence of applying N to crops, Russell (1950) argued that N increases the size of the cells and gives them a thinner wall, hence makes the leaves more succulent and less harsh and that a very low N supply on the other hand gives leaves that are harsh and fibrous, which was observed in this study. It is interesting that he referred to "the excessive development of straw" as harmful secondary effects of N fertilization for it induces a liability of the crop to lodge and may delay the time the wheat comes to maturity.

The importance of N management does not seem to have changed since then, as it is still the most limiting nutrient for crops (Novoa and Loomis, 1981). Concerns over environmental problems caused by N loss, for example nitrate, from the field to surface and ground water have been adding the importance of N issue in agriculture (Anon., 2002b).

7.4 Aspects of Varieties

7.4.1 Introduction of Varieties to the Present Study

Although it was not the primary objective of this study to investigate variety-originated differences in crop performance as to the severity of *Septoria* diseases as well as the pattern of DM and N accumulation, variety was introduced as a factor of the experiment from the second year following the publication by Bayles (1999) that some wheat varieties respond better to strobilurin fungicides compared to others. In her study, Hereward was referred to be relatively less responding to strobilurins compared, for example, to Equinox. It was the reason why Malacca and Equinox were introduced from the second year in this study. Malacca was omitted in the third year partly because it was also a bread-making variety like Hereward and partly due to the fact that the unusual nature of weather conditions prevailed prior to and during Cycle II made it difficult to separate the effects of treatments from those of environment, especially weather in this case. Equinox was continued to be employed in the third year in the attempt of understanding whether variety would be a significant factor in determining the efficacy of fungicide programmes in terms of N fertilization.

7.4.2 Green Leaf Area, Dry Matter Accumulation and Yield

In Cycle II, variety did neither cause interaction in DMW of any plant part at any sampling time throughout the grain fill with respect to fungicide programmes nor difference in grain yield at pre-harvest. It was in Cycle III-C that a few interactions were observed in grain yield between varieties and fungicide programmes. The yield of combine-harvested grains showed that Equinox responded to a mixture of epoxiconazole and trifloxystrobin better than the plots treated with either epoxiconazole alone or a mixture of epoxiconazole and kresoxim-methyl, while, in the case of Hereward, there was no significant difference in grain yield between fungicide programmes. This is similar to the report by Bayles (1999) that a greater yield of Equinox was achieved in response to strobilurin-based fungicide programme compared to conventional fungicide programmes such as triazole. However, the two studies are different when looking at them in more details. In Bayles (1999), the greater yield of Equinox was observed in response to kresoxim-methyl, while in this study, yield was improved in response to a mixture of epoxiconazole and trifloxystrobin rather than a mixture of epoxiconazole and kresoxim-methyl.

As to yield components, Equinox and Hereward performed differently with respect to TGW and HI in Cycle III-C. For both varieties, TGW was greater for the plots treated with fungicide programmes than untreated plots. However, the difference in TGW between fungicide programmes was observed only on Equinox showing a greater TGW for the plots treated with one of the combinations of triazole and strobilurin, i.e. a mixture of epoxiconazole and trifloxystrobin, than those treated with either triazole programme, epoxiconazole alone or the other combination of triazole and strobilurin, a mixture of epoxiconazole and

kresoxim-methyl. There was a relatively strong positive correlation between TGW and HI in Cycle III-C, while no correlation was observed in Cycle III-B where treatments consisted of only fungicide programmes and N rates (data not shown). The difference in HI between the plots treated with a mixture of epoxiconazole and trifloxystrobin and untreated plots as well as the plots treated with epoxiconazole alone was only observed on Equinox. Significance response to the combination of triazole and strobilurin observed on Equinox in TGW and HI seems to indicate that this particular variety is likely to increase the amount of assimilates translocated to grains in response to strobilurins.

7.4.3 Nitrogen Accumulation

In Cycle III-C, grain N concentration was not significantly influenced by fungicide programmes for Hereward. For Equinox, untreated plots showed a significantly greater grain N concentration than untreated plots. In addition, grain N concentration was found to be significantly greater for the plots treated with epoxiconazole alone than those treated with a mixture of epoxiconazole and kresoxim-methyl. A greater N concentration of untreated plots for Equinox would probably be explained by a particularly severe infection of *Septoria* diseases observed in this field experiment (Black, 2003), as *Septoria* diseases are known to reduce TGW (Simon *et al.*, 2002) and actually a significantly lower grain yield was observed for untreated plots in this field experiment. Although a lower grain N concentration observed for the plots treated with a mixture of epoxiconazole and kresoxim-methyl appeared to be indicating a dilution effect of the fungicide programme, there did not seem to be any increase in grain yield to the extent to cause dilution of N. The plots treated with a mixture of epoxiconazole and trifloxystrobin would be more likely to cause dilution, but

it was not the case.

7.4.4 Concluding Remarks of 7.4

As new varieties always come up and replace older ones in reality, it is possible that the gained knowledge on a certain variety may be useful only for a short period. For agronomists, it is probably not good enough to say a particular variety performs well in a particular environmental condition with a particular production package. The accumulation of knowledge that is useful for much longer period could be achieved if the nature of varieties was studied in a manner to elucidate underlying physiological mechanisms no matter whether in response to environment or management factors such as crop protection by fungicides, or both. Only by doing so, would we be able to apply the gained knowledge not only to the current problems but also to new situations.

7.5 Systems Approach and Modeling

7.5.1 *Septoria* diseases

At the canopy level of study such as this, it was considered to be extremely difficult or impossible to quantify the degree of influence of foliar diseases accurately enough to allow the integration of the disease component into the DM and N assimilation processes of the canopy. Even the clear distinction between diseased leaf area and senesced leaf area was difficult by visual assessment. In this study one of the important objectives

was to investigate whether a better crop performance such as higher yield and higher grain N concentration, originated from crop physiological mechanisms rather than the consequence of controlling foliar diseases to different degrees, and also if it would be obtained following the application of strobilurin fungicide programmes compared to a triazole fungicide programme. Unless both triazole and strobilurin fungicide programmes completely controlled foliar diseases, it would be impossible to make a comparison between fungicide programmes on pure physiological grounds as to crop performance such as yield and grain N concentration. Green leaf area is a more reliable in practical sense to study DM accumulation (Gaunt, 1995), which enables the conceptual simplification of wheat-*Septoria* pathosystem such as this study. Only by a simplified way is it possible to study such a system in a quantitative manner.

7.5.2 Green Leaf Area, Dry Matter Accumulation and Yield

As demonstrated in Chapter 3 and Chapter 6, GLAI is essential in estimating light interception and DM accumulation. Although only leaf blade was considered in this study to enable faster processing of the sampled plants, ideally leaf sheath should be included in the calculation of GLAI. Green ears might be making a significant contribution to carbon assimilation as well.

Theoretically DM accumulation can be calculated by subtracting respiration from gross carbon assimilation. As measurements of the two processes throughout the growing season is not possible in most of the cases, measurement of DMW of sampled plants from a known area is used to estimate biomass accumulation knowing that this way of estimation is subjected to various types of errors. Loss of biomass, for example,

by defoliation and predatory attack, could not be assessed, therefore the criticism given to Gallagher *et al.* (1975) by Bidinger *et al.* (1977) applies also to this study. However, this is the most convenient method of estimation and can be used for comparison between treatments applied to the crop under otherwise the same growing condition. As is often the case with this type of study, roots were not sampled and therefore ignored assuming that the percentage of contribution of roots is acceptably similar between treatments.

Pearson's correlation analysis performed on the rate of increase in grain DMW and the rate of decrease in vegetative DM during grain fill revealed a striking contrast between Cycle II and Cycle III-B with respect to translocation of assimilates. It was indicated that a greater portion of assimilates came from vegetative reserves in Cycle II compared to Cycle III-B.

GNA was neither affected by *Septoria* diseases nor fungicide programmes, while TGW was. Prew *et al.* (1985) reported a case where the number of ears and the number of grains per ear were increased by fungicides when particularly severe level of foliar diseases was observed. Except for extreme conditions where GNA, in other words, sink size is greatly affected by particularly severe level of *Septoria* diseases, it is not likely that neither *Septoria* diseases nor fungicide programmes could influence grain yield to the same magnitude as N which determines the sink size.

7.5.3 Concluding Remarks of 7.5

The objectives of Chapter 6 were to estimate, crudely, biomass accumulation from leaf N concentration and

GLAI. It was implied that the exclusion of leaf sheath and ears could cause a significant underestimation of biomass accumulation. Numerically a difference was simulated in biomass accumulation between fungicide programmes, but it was much smaller than that between N rates, the same result observed with the measured data (see Chapter 3). Despite that the attempt of modeling in this study was very crude and was susceptible to errors of various sources, simulating numerical differences in biomass accumulation as small as those caused by fungicide programmes seemed very difficult. Maybe efforts need to be made to model DM translocation from the vegetative tissues to the grains of the strobilurin-treated wheat crops, which was statistically detectable in this study. There is no doubt that parameters are crucial in modeling but it is not always possible to take sufficient data that enable derivation of the parameters especially when the effects of a number of treatments are compared in factorial design. Although only biomass accumulation was considered by modeling approach in this study, it would be of interest to make an attempt to model the N component of strobilurin-treated wheat crops especially in relation to the DM component.

7.6 Future Work

Three points are considered to be worthwhile testing in further experiments, i.e. the effects and implications of applying strobilurin fungicides on 1) crop N uptake, 2) crop water use and water use efficiency and 3) morphological and physiological properties of leaves.

1) Crop N uptake

It was implied from this study that the increased grain N content observed with the plots applied with a combination of triazole and strobilurin, i.e. a mixture of epoxiconazole and trifloxystrobin is likely to be attributable to an improved efficiency in N translocation from vegetative organs to grains rather than an increased N uptake by the crop, which might happen depending on the season but with less consistency compared to the increase in N translocation. It has to be noted, however, that the range of N rates tested in this study was rather limited, as the maximum N rate followed the recommendations by MAFF (Anon., 2000) except for Cycle III-C where the higher N rate (i.e. 200 kg ha^{-1}) could have been supraoptimum as has been discussed in section '4.4.2 N Harvest Index (NHI)' in Chapter 4. Under the condition of relatively limited range of applied N, there might remain the possibility that the ability of roots of the strobilurin-treated wheat crops might not have been fully expressed. Measuring N uptake of the strobilurin-treated wheat crop under a wider range of soil N than that tested in this study might help us understand the lack of consistency in N uptake observed with the strobilurin-treated wheat crop (Clark and Jones, 1999; Bryson, 2000; Jones *et al.*, 2001; Anon., 2003a). In order to enable more accurate assessment of N uptake by the strobilurin-treated wheat crops than that achieved in the field, it would be preferable that the plant be grown in pots of either hydroponics or the type of soils which contain a very limited level of organic N where the inflows of N could be controlled. The size of the pots should be preferably large not to restrict the growth of the root too severely. Different levels of N should be supplied to the crops including the extremes both low and high levels. The crops should be sampled including roots at maturity to assess the amount of N uptake. The idea is formulated into the 'Null hypothesis to be tested in the future work 1.' in the last part of this section.

2) Crop water use and water use efficiency (WUE)

Following the observation made by Sumi and Katayama (2001) that dwarf isogenic lines of sorghum, soybean and rice tend to use less water to achieve the same level of the yield as the tall lines, in other words, improved water use efficiency (WUE), it was considered that the shorter plant height observed in this study following the application of a mixture of epoxiconazole and trifloxystrobin might be an indication that less water is transpired by the crop treated with this fungicide programme compared to those treated with either epoxiconazole alone or a mixture of epoxiconazole and kresoxim-methyl (Null hypothesis to be tested in the future work 2.). Unless lysimeters are available, the amount of transpired water can be estimated in a pot experiment by weighing each pot before and after every watering, the method employed by Sumi and Katayama (2001).

3) Morphological and physiological properties of leaves

While carrying out the field experiments in this study, a change in the surface property as well as the shape of a leaf was observed as it became shaded by newly formed leaves. Presumably it was the transition phase from sun leaf to shade leaf. The impact of light condition on a plant canopy with respect to productivity is well known. As has been mentioned in the introduction of Chapter 4, Ottman and Welch (1988) reported a delayed senescence followed by increased DM and N accumulations in response to the supplemental radiation applied to the lower part of the maize canopy. Accelerated leaf senescence in lower part of the canopy characterized with poor light condition was considered to be triggered by low R (red light) : FR (far-red radiation) ratios (Rousseaux *et al.*, 1996), one of the signals for the plants to detect the surrounding

environment (Ballaré *et al.*, 1995). Considering that one of the main effects of applying strobilurin fungicides is to delay leaf senescence or, in other words, to maintain green leaf and that this study suggested a possible morphological and physiological change in leaf properties caused by the application of fungicides as observed with SLA, it would be interesting to investigate the interactive effects of light conditions and fungicides on leaf morphological and physiological properties with particular reference to leaf senescence (Null hypothesis to be tested in the future work 3.). A pot experiment of factorial design where fungicide programmes and R : FR ratios are the treatments is suggested for the future study.

Null Hypothesis to be tested in the Future Work

1. The use of a strobilurin fungicide in a disease control programme in wheat does not affect N accumulation under conditions of extreme soil N content.

2. The use of a strobilurin fungicide in a disease control programme in wheat does not affect the size of the canopy hence the amount of water transpired during the growing season as well as water use efficiency (WUE).

3. There is no synergistic effect between the use of strobilurin fungicides and R : FR ratios of irradiance on the process of leaf senescence and morphological and physiological leaf properties.

Appendix

Appendix 1 (a) Layout of field experiments

Cycle I (1999/2000)

	Block 3								
	3	7	2	8	5	11	9	5	12
Block 2	9	6	4	12	1	10	4	2	7
	7	9	5	4	8	10	6	1	8
Block 1	2	12	11	6	3	1	10	3	11

Cycle II (2001)

Block 3	14	8	16	2	24	1	19	13	5	17	7	6	12	9	3	22	20	11	23	18	15	10	4	21
Block 2	1	21	19	10	12	6	14	13	17	15	3	11	18	23	16	5	9	24	20	7	8	22	4	2
Block 1	5	3	23	17	21	8	20	13	10	1	24	2	14	7	18	15	11	16	6	19	12	9	4	22

Cycle III-A

	Block 1		Block 2		Block 3		Block 4	
Block 4	3	2	5	1	7	4	8	6
Block 3	1	5	6	4	8	2	7	3
Block 2	6	7	2	8	5	3	1	4
Block 1	8	4	3	7	1	6	5	2

Cycle III-B

Block 4	4S	4H	5S	5H	3S	3H	7S	7H	6S	6H	9S	9H	8S	8H	1S	1H	2S	2H
Block 3	7S	7H	3S	3H	5S	5H	1S	1H	9S	9H	2S	2H	8S	8H	4S	4H	6S	6H
Block 2	4S	4H	2S	2H	6S	6H	9S	9H	1S	1H	8S	8H	3S	3H	5S	5H	7S	7H
Block 1	9S	9H	1S	1H	6S	6H	7S	7H	4S	4H	3S	3H	5S	5H	8S	8H	2S	2H

S: Sampling plot
H: Harvest plot

Cycle III-C

	Block 1				Block 2				Block 3				Block 4			
Block 4	10	3	5	2	13	4	7	11	8	12	16	15	1	6	9	14
Blcok 3	14	4	15	7	12	1	5	10	11	6	9	3	2	16	8	13
Block 2	1	16	11	8	14	2	9	6	13	5	4	10	3	7	12	15
Block 1	12	9	13	6	15	8	3	16	2	14	7	1	11	4	10	5

Appendix 1 (b)

Treatments corresponding to the numbers in Appendix 1 (a)

Table A. 1(b)-1 Treatments in Cycle I

Number	*Fungicide programme*	N rate (kg ha^{-1})
1	untreated	0
2	untreated	100
*3**	untreated	140
4	epoxiconazole (Opus)	0
5	epoxiconazole (Opus)	100
6	epoxiconazole (Opus)	140
7	epoxiconazole + kresoxim-methyl (Landmark)	0
8	epoxiconazole + kresoxim-methyl (Landmark)	100
9	epoxiconazole + kresoxim-methyl (Landmark)	140
10	epoxiconazole + trifloxystrobin (Opus + Twist)	0
11	epoxiconazole + trifloxystrobin (Opus + Twist)	100
12	epoxiconazole + trifloxystrobin (Opus + Twist)	140

* missing treatment

As to fungicide programmes, only product names will be listed in the following tables.

Table A. 1(b)-2 Treatments in Cycle II

Number	*Variety*	*Fungicide Programme*	N rate (kg ha^{-1})
1	Hereward	untreated	90
2	Hereward	untreated	130
3	Hereward	Opus	90
4	Hereward	Opus	130
5	Hereward	Landmark	90
6	Hereward	Landmark	130
7	Hereward	Opus + Twist	90
8	Hereward	Opus + Twist	130
9	Malacca	untreated	90
10	Malacca	untreated	130
11	Malacca	Opus	90
12	Malacca	Opus	130
13	Malacca	Landmark	90
14	Malacca	Landmark	130
15	Malacca	Opus + Twist	90
16	Malacca	Opus + Twist	130
17	Equinox	untreated	90
18	Equinox	untreated	130
19	Equinox	Opus	90
20	Equinox	Opus	130
21	Equinox	Landmark	90
22	Equinox	Landmark	130
23	Equinox	Opus + Twist	90
24	Equinox	Opus + Twist	130

Table A. 1(b)-3 Treatments in Cycle III-A

Number	*Seed rate (m^{-2})*	N rate (kg ha^{-1})
1	100	0
2	100	75
3	100	150
4	100	250
5	400	0
6	400	75
7	400	150
8	400	250

Table A. 1(b)-4 Treatments in Cycle III-B

Number	*Fungicide Programme*	N rate (kg ha^{-1})
1	untreated	0
2	untreated	100
3	untreated	140
4	Opus	0
5	Opus	100
6	Opus	140
7	Opus + Twist	0
8	Opus + Twist	100
9	Opus + Twist	140

Table A. 1(b)-5 Treatments in Cycle III-C

Number	*Variety*	*Fungicide Programme*	*N rate (kg ha^{-1})*
1	Hereward	untreated	100
2	Hereward	untreated	200
3	Hereward	Opus	100
4	Hereward	Opus	200
5	Hereward	Landmark	100
6	Hereward	Landmark	200
7	Hereward	Opus + Twist	100
8	Hereward	Opus + Twist	200
9	Equinox	untreated	100
10	Equinox	untreated	200
11	Equinox	Opus	100
12	Equinox	Opus	200
13	Equinox	Landmark	100
14	Equinox	Landmark	200
15	Equinox	Opus + Twist	100
16	Equinox	Opus + Twist	200

Appendix 2

Husbandry of Field Experiments

Table A. 2-1 Field Husbandry of Cycle I

Date	*Operation*	*Notes*
14/Oct/1999	Sowing	No fertilization
		350 seeds/m2
13/Mar/2000	Herbicide Application	Capture: 6.5 l/ha
	Insecticide Application	Cypermethrin: 0.25 l/ha
	Nutrient Application	Profol 500: 7 l/ha
		Coptrel: 0.5 l/ha
17/Mar/2000	Nutrient Application	MgS: 75 kg/ha
23/Mar/2000	N Fertilizer Application (1^{st})	2 levels
		0, 40 kg/ha
30/Mar/2000	Fungicide Application (1^{st})	4 treatments
14/April/2000	N Fertilizer Application (2^{nd})	3 levels
		0, 60, 100 kg/ha
24/May/2000	Fungicide Application (2^{nd})	4 treatments
25/Aug/2000	Combine-Harvest	

Table A. 2-2 Field Husbandry of Cycle H

Date	*Operation*	*Notes*
12/Jan/2001	Sowing	No fertilization
		350 seeds/m2
30/Apr/2001	Herbicide Application	Panther: 2 l/ha
	Insecticide Application	Cyperkill: 0.25 l/ha
	Nutrient Application	Manifol: 1 l/ha
18/May/2001	N Fertilizer Application (1^{st})	1 level
		40 kg/ha
21/May/2001	Fungicide Application (1^{st})	4 treatments
22/May/2001	Herbicide Application	Ally: 20 g/ha
		Starane 2: 0.5l/ha
		Cheetah super: 1 l/ha
	Nutrient Application	Manganese 1 l/ha
05/Jun/2001	N Fertilizer Application (2^{nd})	2 levels
		50, 90kg/ha
14/Jun/2001	Fungicide Application (2^{nd})	4 treatments
28/Aug/2001	Combine-Harvest	

Table A. 2-3 (a) Field Husbandry of Cycle III-A

Date	*Operation*	*Notes*
26/Sep/2001	Sowing	No fertilization
		100, 400 seeds/m^2
14/Nov/2001	Herbicide Application	Trooper: 2 l/ha
		Ardent: 0.5 l/ha
		Panther: 0.5 l/ha
	Insecticide Application	Toppel: 0.25 l/ha
07/Mar/2002	Nutrient Application	S
	N Fertilizer Application (1^{st})	2 levels
		0, 40 kg/ha
25/Mar/2002	PGR Application	Moddus: 0.2 l/ha
		CCC: 1.25 l/ha
03/Apr/2002	N Fertilizer Application (2^{nd})	4 levels
		0, 35, 110, 210 kg/ha
16/May/2002	Herbicide Application	Starane 2: 0.7 l/ha
		Ally: 15 g/ha
	Nutrient Application	Manifol: 1 l/ha
16/Aug/2002	Combine-Harvest	

Table A. 2-3 (b) Field Husbandry of Cycle III-B

Date	*Operation*	*Notes*
26/Sep/2001	Sowing	No fertilization
		100, 400 seeds/m^2
14/Nov/2001	Herbicide Application	Trooper: 2 l/ha
		Ardent: 0.5 l/ha
		Panther: 0.5 l/ha
	Insecticide Application	Toppel: 0.25 l/ha
06/Mar/2002	N Fertilizer Application (1^{st})	2 levels
		0, 40 kg/ha
19/Mar/2002	Fungicide Application (1^{st})	3 treatments
	PGR Application	Moddus: 0.2 l/ha
		CCC: 1.25 l/ha
02/Apr/2002	N Fertilizer Application (2^{nd})	3 levels
		0, 60, 100 kg/ha
16/May/2002	Herbicide Application	Starane 2: 0.7 l/ha
		Ally: 15 g/ha
	Nutrient Application	Manifol: 1 l/ha
26/May/2002	Fungicide Application	3 treatments
16/Aug/2002	Combine-Harvest	

Table A. 2-3 (c) Field Husbandry of Cycle III-C

Date	Operation	Notes
26/Sep/2001	Sowing	No fertilization
		100, 400 seeds/m^2
14/Nov/2001	Herbicide Application	Trooper: 2 l/ha
		Ardent: 0.5 l/ha
		Panther: 0.5 l/ha
	Insecticide Application	Toppel: 0.25 l/ha
05/Mar/2002	N Fertilizer Application (1^{st})	1 level
		40 kg/ha
11/Mar/2002	S Fertilizer Application	
13/Mar/2002	Fungicide Application (1^{st})	4 treatments
	PGR Application	Moddus: 0.2 l/ha
		CCC: 1.25 l/ha
28/Mar/2002	N Fertilizer Application (2^{nd})	2 levels
		60, 160 kg/ha
16/May/2002	Herbicide Application	Starane 2: 0.7 l/ha
		Ally: 15 g/ha
	Nutrient Application	Manifol: 1 l/ha
31/May/2002	Fungicide Application	4 treatments
16/Aug/2002	Combine-Harvest	

Appendix 3

Date of Samplings and Foliar Disease Assessments

Sampling	*Cycle I*		*Cycle II*		*Cycle III-A*		*Cycle III-B*		*Cycle III-C*	
	B	**E**	**B**	**E**	**B**	**E**	**B**	**E**	**B**	**E**
1st	15/05/00	17/05/00	29/06/01	03/07/01	23/05/02	24/05/02	08/04/02	12/04/02	-	-
	(214)	(216)	(168)	(172)	(239)	(240)	(194)	(198)		
2nd	30/05/00	02/06/00	17/07/01	24/07/01	04/06/02	05/06/02	21/05/02	28/05/02	-	-
	(229)	(232)	(186)	(193)	(251)	(252)	(237)	(244)		
3rd	11/06/00	16/06/00	-	-	25/06/02	26/06/02	08/06/02	17/06/02	-	-
	(241)	(246)			(272)	(273)	(255)	(264)		
4th	27/06/00	29/06/00	-	-	-	-	22/06/02	11/07/02	-	-
	(257)	(259)					(269)	(288)		
5th	11/07/00	13/07/00	-	-	-	-	18/07/02	25/07/02	-	-
	(271)	(273)					(295)	(302)		
6th	24/07/00	26/07/00	-	-	-	-	-	-	-	-
	(284)	(286)								
Pre-Harvest	01/08/00	03/08/00	14/08/01	19/08/01	-	-	03/08/02	15/08/02	06/08/02	09/08/02
	(292)	(294)	(214)	(219)			(311)	(323)	(314)	(317)
Harvest	25/08/00	-	28/08/01		-	-	16/08/02	-	16/08/02	-
	(316)		(228)				(324)		(324)	
Additional (LAI)	05/06/00	07/06/00	-	-	-	-	-	-	-	-
	(235)	(237)								

B: Beginning date of each sampling

E: End date of each sampling

The number between bracket shows days after sowing (DAS) for each field experiment

Appendix 4

Date of Crop Growth Stage for Each Field Experiment

Field Experiment	*Cycle I*	*Cycle II*			*Cycle III-A*	*Cycle III-B*	*Cycle III-C*	
Variety	**H**	**H**	**M**	**E**	**H**	**H**	**H**	**E**
Sowing (DAS = 0)	14/10/99	12/01/01	12/01/01	12/01/01	26/09/01	26/09/01	26/09/01	26/09/01
Double Ridge	-	28/04/01	26/04/01	26/04/01	-	-	-	-
		(106)	(104)	(104)				
GS 30/31	23/03/00	18/05/01	18/05/01	18/05/01	07/03/01	05/03/01	05/03/01	05/03/01
	(161)	(126)	(126)	(126)	(162)	(160)	(160)	(160)
GS 32	14/04/00	05/06/01	05/06/01	05/06/01	03/04/02	02/04/02	28/03/02	28/03/02
	(183)	(144)	(144)	(144)	(189)	(188)	(183)	(183)
GS 39	23/05/00	14/06/01	14/06/01	14/06/01	-	-	-	-
	(222)	(153)	(153)	(153)				
GS 65	13/06/00	27/06/01	27/06/01	47/06/01	10/06/02	10/06/02	10/06/02	10/06/02
	(243)	(166)	(166)	(166)	(257)	(257)	(257)	(257)
Harvest	25/08/00	28/08/01	28/08/01	28/08/01	16/08/02	16/08/02	16/08/02	16/08/02
	(316)	(228)	(228)	(228)	(324)	(324)	(324)	(324)

H: Hereward, M: Malacca, E: Equinox

The number between bracket shows days after sowing (DAS)

Appendix 5

Leaf Separation for DMW determination and N analysis

Table A. 5-1 (a) Leaf separation at samplings for DMW in Cycle I

1st	*2nd*	*3rd (Anthesis)*	*4th*	*5th*	*6th (Pre-Harvest)*
G Leaf	G Leaf	G Leaf 1	G Leaf 1	G Leaf 1	G Leaf
		G non-Leaf 1	G non-Leaf 1	G non-Leaf 1	
Sen Leaf	Sen Leaf	Sen Leaf	Sen Leaf	Sen Leaf	Sen Leaf

G: green

Sen: senesced

Table A. 5-1 (b) Leaf Separation at samplings for N analysis in Cycle I

1st	*2nd*	*3rd (Anthesis)*	*4th*	*5th*	*6th (Pre-Harvest)*
G Leaf	G Leaf	G Leaf	G Leaf	G Leaf	Total Leaf
Sen Leaf	Sen Leaf	Sen Leaf	Sen Leaf	Sen Leaf	

G: green

Sen: senesced

Table A. 5-2 Leaf separation at samplings for DMW and N analysis in Cycle II

1st	*2nd*	*3rd (Pre-Harvest)*
Upper Leaf	Upper Leaf	Upper Leaf
Lower Leaf	Lower Leaf	Lower Leaf

Table A. 5-3 (a) Leaf separation at samplings for DMW in Cycle III-B

1st	*2nd*	*3rd (Anthesis)*	*4th*	*5th*	*6th (Pre-Harvest)*
G Leaf	Leaf 1	Leaf 1	Leaf 1	Leaf 1	Sen Leaf
	Leaf 2	Leaf 2	Leaf 2	Leaf 2	
	Low G Leaf	Low G Leaf	Low G Leaf	Low G Leaf	
Sen Leaf	Low Sen Leaf	Low Sen Leaf	Low Sen Leaf	Low Sen Leaf	

G: green

Sen: senesced

Low: lower

Table A. 5-3 (b) Leaf separation at samplings for N analysis in Cycle III-B

1st	*2nd*	*3rd (Anthesis)*	*4th*	*5th*	*6th (Pre-Harvest)*
G Leaf	Leaf 1	Leaf 1	Leaf 1	Leaf 1	Sen Leaf
	Leaf 2	Leaf 2	Leaf 2	Leaf 2	
	Low G Leaf	Low G Leaf	Low G Leaf	Low Leaf	
Sen Leaf	Low Sen Leaf	Low Sen Leaf	Low Sen Leaf		

G: green

Sen: senesced

Low: lower

Appendix 6

LAI in Cycle III-B

Table A. 6-1 (a) LAI of flag leaf in Cycle III-B

Fungicide Programmes	*N rates (kg ha^{-1})*	*DAS*			
		237-244 (GS 49)	*255-264 (GS 65)*	*269-288*	*295-302*
untrt	*0*	0.47	0.43	0.36	0.04
	100	0.98	0.71	0.56	0.03
	140	1.06	0.90	0.68	0.12
epoxi	*0*	0.41	0.36	0.34	0.06
	100	0.88	0.88	0.69	0.20
	140	1.07	0.94	0.75	0.28
epoxi + triflo	*0*	0.42	0.41	0.43	0.13
	100	0.96	0.91	0.79	0.31
	140	1.17	1.06	0.87	0.63
P value	*Fungicide*	= 0.406	= 0.049	= 0.030	< 0.001
	Nitrogen	< 0.001	< 0.001	< 0.001	< 0.001
	Interaction	= 0.610	= 0.161	= 0.792	= 0.065
L.S.D.	*Fungicide*	NS	0.09	0.12	0.12
	Nitrogen	0.10	0.09	0.12	0.12
	Interaction	NS	NS	NS	NS
CV %		14.1	14.2	23.2	70.1

untrt: untreated; epoxi: epoxiconazole; triflo: trifloxystrobin

Table A. 6-1 (b) LAI of second leaf in Cycle III-B

Fungicide Programmes	*N rates (kg ha^{-1})*	*DAS*			
		237-244 (GS 49)	*255-264 (GS 65)*	*269-288*	*295-302*
untrt	*0*	0.66	0.53	0.34	0.03
	100	1.38	0.98	0.66	0.01
	140	1.45	1.16	0.78	0.02
epoxi	*0*	0.57	0.46	0.39	0.06
	100	1.26	1.14	0.86	0.15
	140	1.43	1.17	0.93	0.16
epoxi + triflo	*0*	0.58	0.51	0.52	0.13
	100	1.34	1.17	1.04	0.25
	140	1.54	1.34	1.11	0.40
P value	*Fungicide*	= 0.460	= 0.057	= 0.003	< 0.001
	Nitrogen	< 0.001	< 0.001	< 0.001	= 0.019
	Interaction	= 0.867	= 0.183	= 0.853	= 0.105
L.S.D.	*Fungicide*	NS	NS	0.16	0.08
	Nitrogen	0.14	0.17	0.16	0.08
	Interaction	NS	NS	NS	NS
CV %		14.3	12.1	25.9	72.6

untrt: untreated; epoxi: epoxiconazole; triflo: trifloxystrobin

Table A. 6-1 (c) LAI of lower leaves in Cycle III-B

Fungicide	*N rates*	*DAS*			
Programmes	*(kg ha^{-1})*	*237-244 (GS 49)*	*255-264 (GS 65)*	*269-288*	*295-302*
untrt	*0*	1.72	0.62	0.12	0.004
	100	3.09	1.23	0.27	0.003
	140	3.36	1.34	0.43	0.005
epoxi	*0*	1.50	0.67	0.31	0.035
	100	3.00	1.51	0.48	0.035
	140	3.30	1.67	0.51	0.037
epoxi + triflo	*0*	1.60	0.88	0.66	0.097
	100	2.94	1.54	0.79	0.081
	140	3.48	1.79	0.92	0.104
P value	*Fungicide*	= 0.680	= 0.002	< 0.001	< 0.001
	Nitrogen	< 0.001	< 0.001	= 0.004	= 0.841
	Interaction	= 0.912	= 0.656	= 0.923	= 0.978
L.S.D.	*Fungicide*	NS	0.17	0.14	0.033
	Nitrogen	0.30	0.17	0.14	NS
	Interaction	NS	NS	NS	NS
CV %		13.3	16.6	33.4	86.5

untrt: untreated; epoxi: epoxiconazole; triflo: trifloxystrobin

Appendix 7

Interactions in Dry Matter Weight

Table A. 7-1 (a) Interactions in DMW of lower stem between fungicide programmes and N rates in Cycle II

DAS	*168 – 172*		*186 – 193*	
Fungicide Programmes	*90 kg N ha^{-1}*	*130 kg N ha^{-1}*	*90 kg N ha^{-1}*	*130 kg N ha^{-1}*
untrt	2050	1980	1580	1710
epoxi	2020	1940	1760	1650
epoxi + kreso	1910	2240	1580	1850
epoxi + triflo	1960	1870	1640	1590
P value	= 0.019		= 0.034	
L.S.D.	210		190	
CV %	11.3		12.2	

untrt: untreated; epoxi: epoxiconazole; kreso: kresoxim-methyl; triflo: trifloxystrobin

Table A. 7-2 (b) Interactions in DMW of lower leaf between fungicide programmes and N rates in Cycle II

DAS	*168 – 172*		*186 – 193*	
Fungicide Programmes	*90 kg N ha^{-1}*	*130 kg N ha^{-1}*	*90 kg N ha^{-1}*	*130 kg N ha^{-1}*
untrt	540	520	460	500
epoxi	540	540	510	500
epoxi + kreso	500	620	460	560
epoxi + triflo	550	590	490	510
P value	= 0.050		= 0.024	
L.S.D.	70		50	
CV %	14.0		11.1	

untrt: untreated; epoxi: epoxiconazole; kreso: kresoxim-methyl; triflo: trifloxystrobin

Appendix 8

TGW of Combine-Harvested Grains

Table A. 8-1 (a) Thousand Grain Weight (TGW) (@ 100 % DM) of combine-harvested grains for varieties

Varieties	*Cycle I*	*Cycle III-B*	*Cycle III-C*
Hereward	46.5	43.8	42.9
Equinox	-	-	44.3
P value	-	-	< 0.001
L.S.D.	-	-	0.70
CV %	2.2	1.9	3.2

Table A. 8-1 (b) Thousand Grain Weight (TGW) (@ 100 % DM) of combine-harvested grains for fungicide programmes

Fungicide Programmes	*Cycle I*	*Cycle III-B*	*Cycle III-C*
untrt	45.8	42.6	40.0
epoxi	46.5	44.0	44.0
epoxi + kreso	47.0	-	44.8
epoxi + triflo	46.7	44.9	45.7
P value	= 0.136	< 0.001	< 0.001
L.S.D.	NS	0.71	0.99
CV %	2.2	1.9	3.2

untrt: untreated; epoxi: epoxiconazole; kreso: kresoxim-methyl; triflo: trifloxystrobin

Table A. 8-1 (c) Thousand Grain Weight (TGW) (@ 100 % DM) of combine-harvested grains for N rates

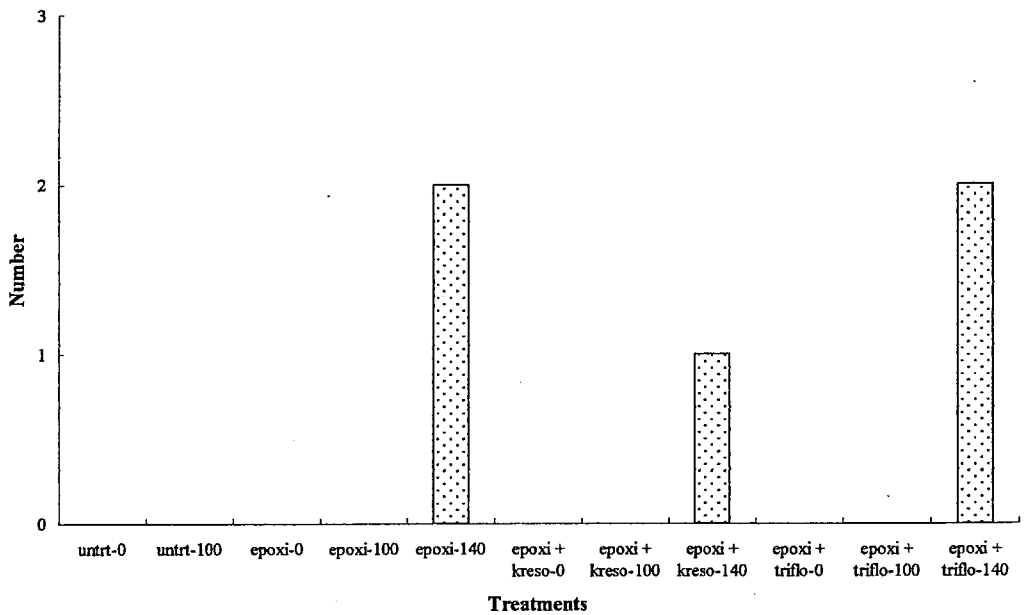
N rates (kg ha^{-1})	*Cycle I*	*Cycle III-B*	*Cycle III-C*
0	46.9	45.0	-
100	46.1	43.6	43.8
140	46.4	42.9	-
200	-	-	43.4
P value	NS	< 0.001	NS
L.S.D.	-	0.71	-
CV %	2.2	1.9	3.2

Appendix 9 (a)

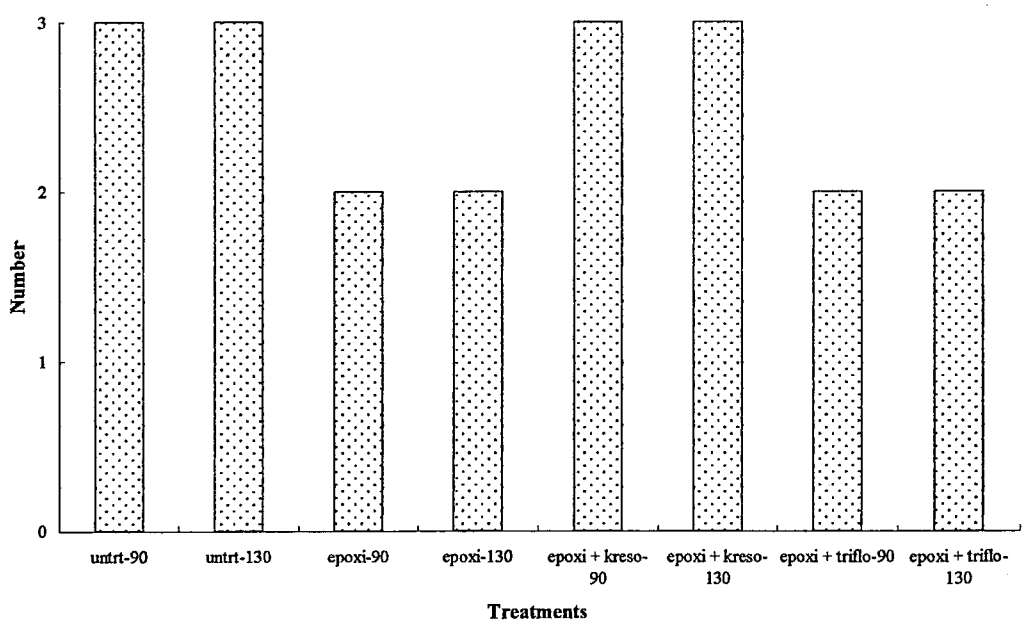
The number of the plots that met the requirement of grain N concentration for bread-making (Manually-Harvested Grains)

untrt: untreated; epoxi: epoxiconazole; kreso: kresoxim-methyl; triflo: trifloxystrobin

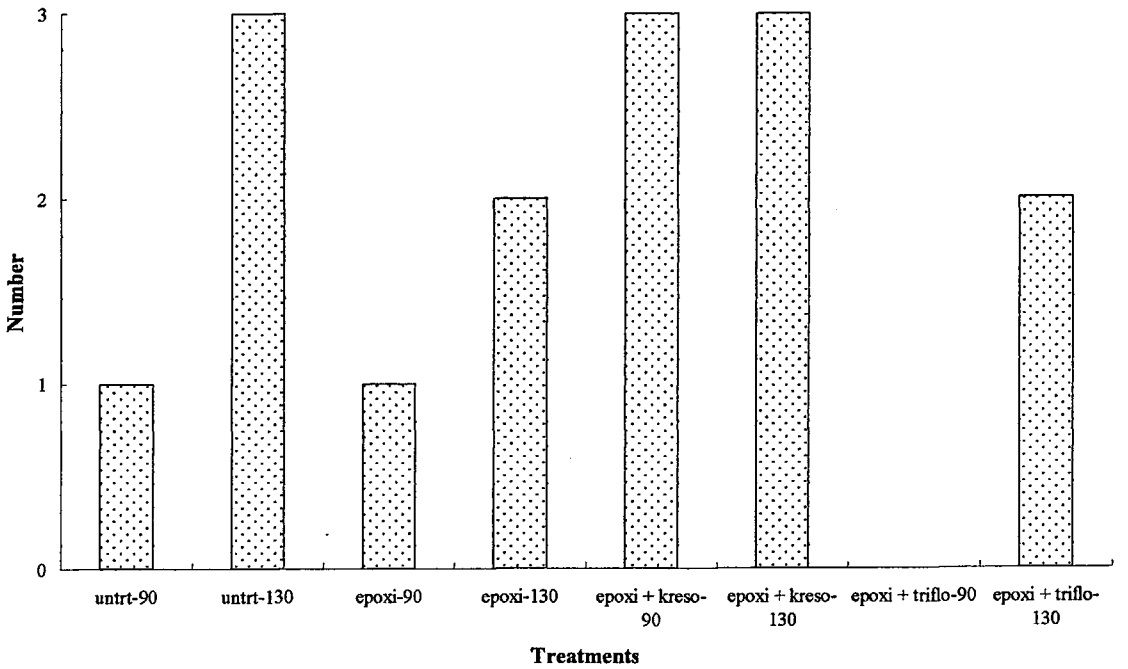
untrt-0: untreated + 0 kg N ha^{-1}



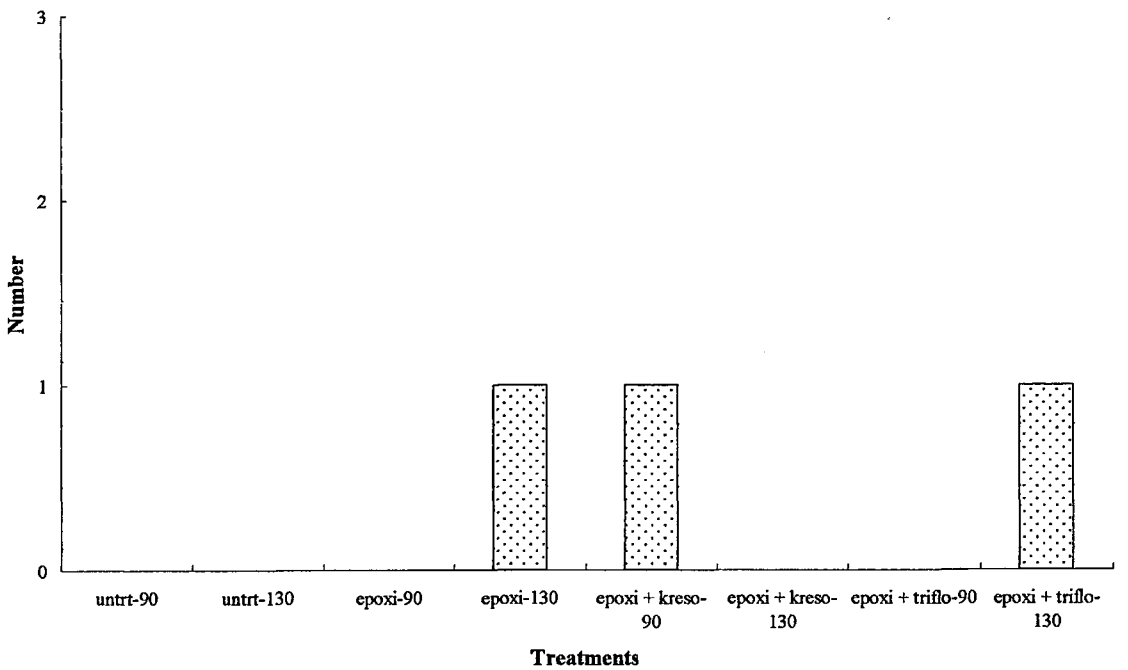
(i) Cycle I – Hereward (max = 3)



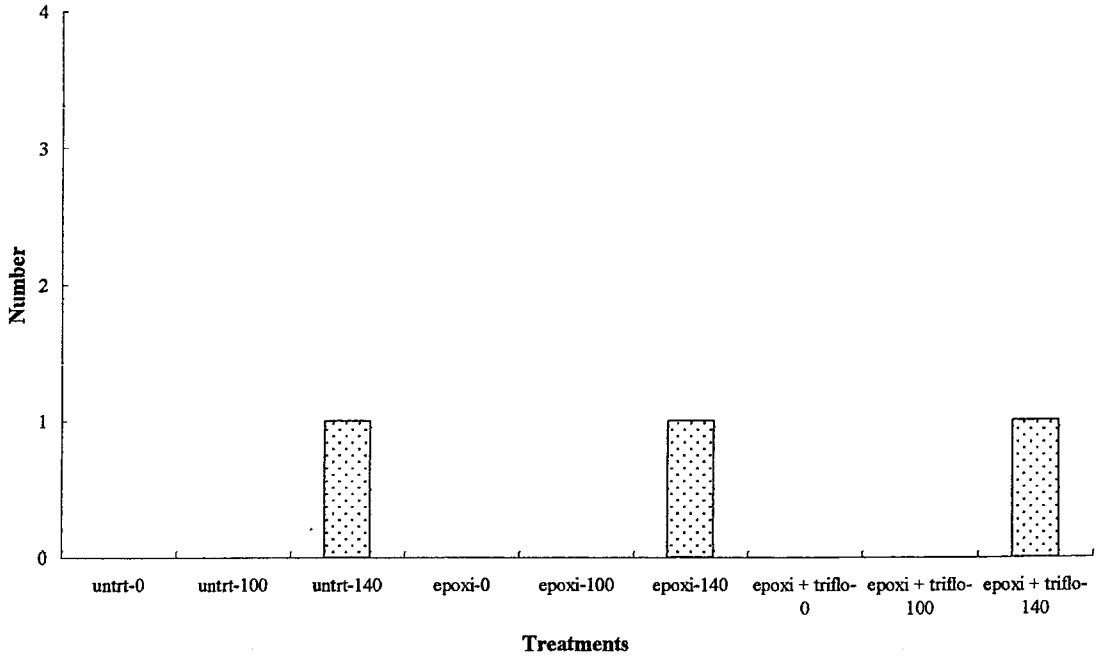
(ii) Cycle II – Hereward (max = 3)



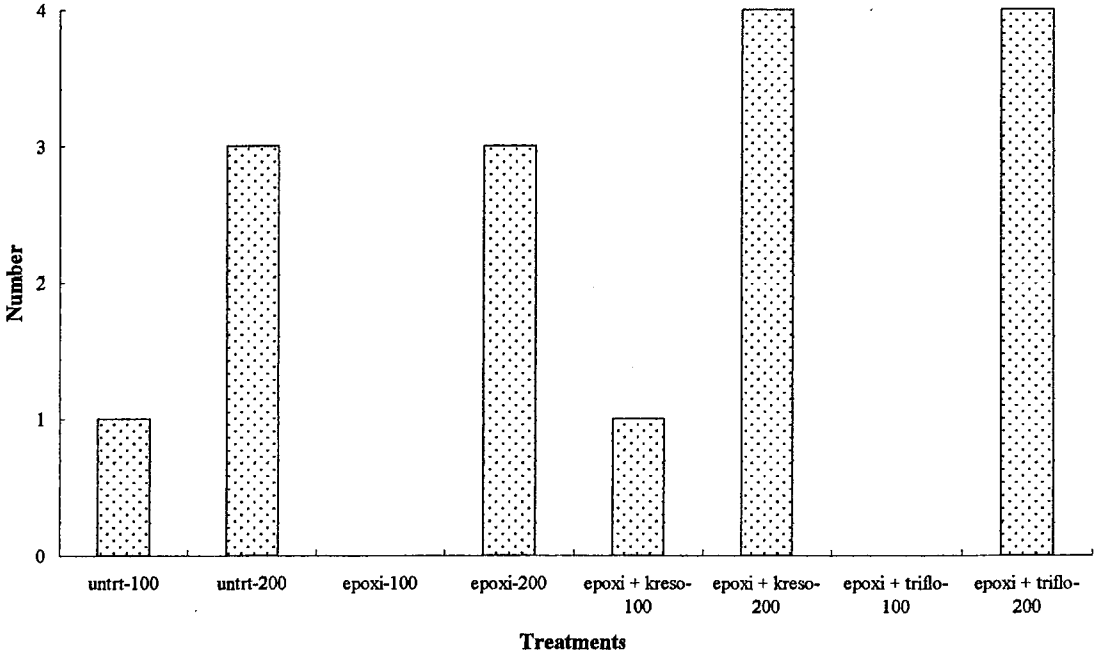
(iii) Cycle II – Malacca (max = 3)



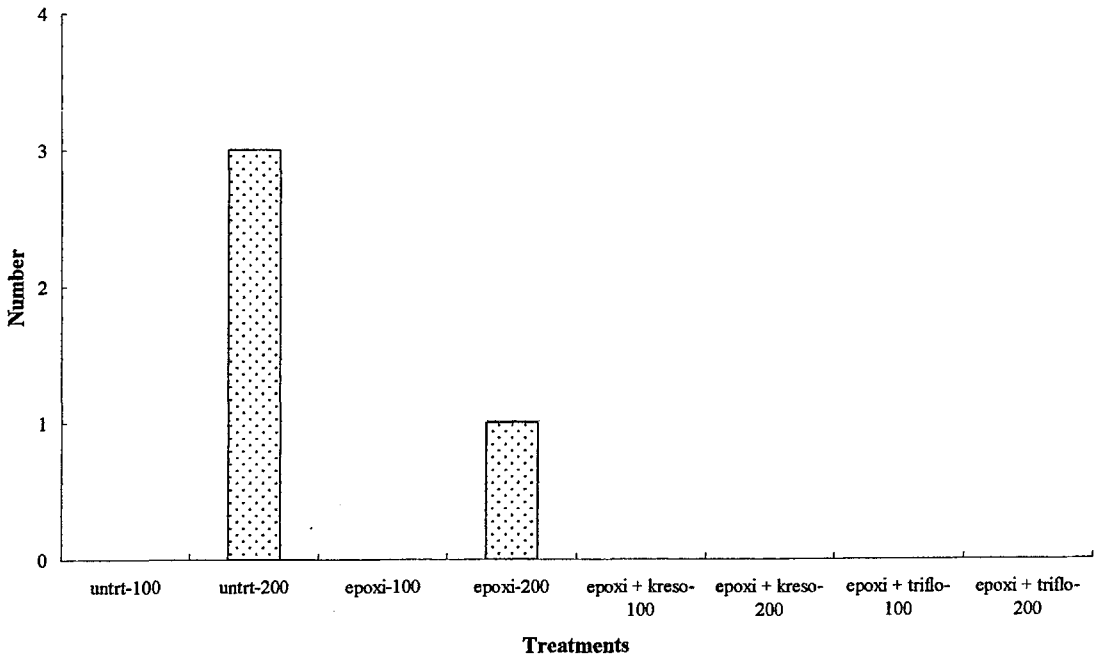
(iv) Cycle II – Equinox (max = 3)



(v) Cycle III-B – Hereward (max = 4)



(vi) Cycle III-C – Hereward (max = 4)

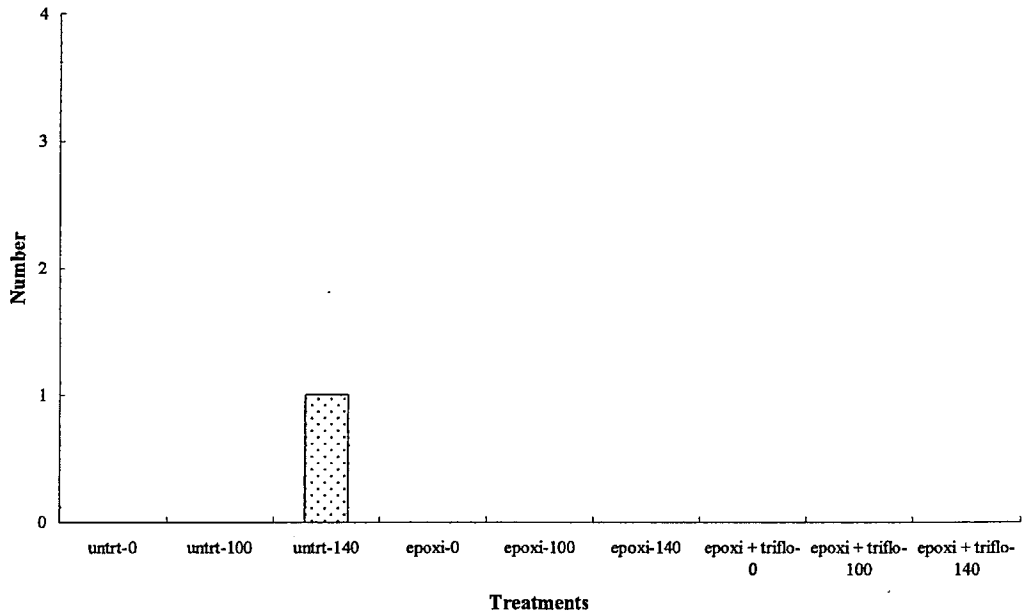


(vii) Cycle III-C – Equinox (max = 4)

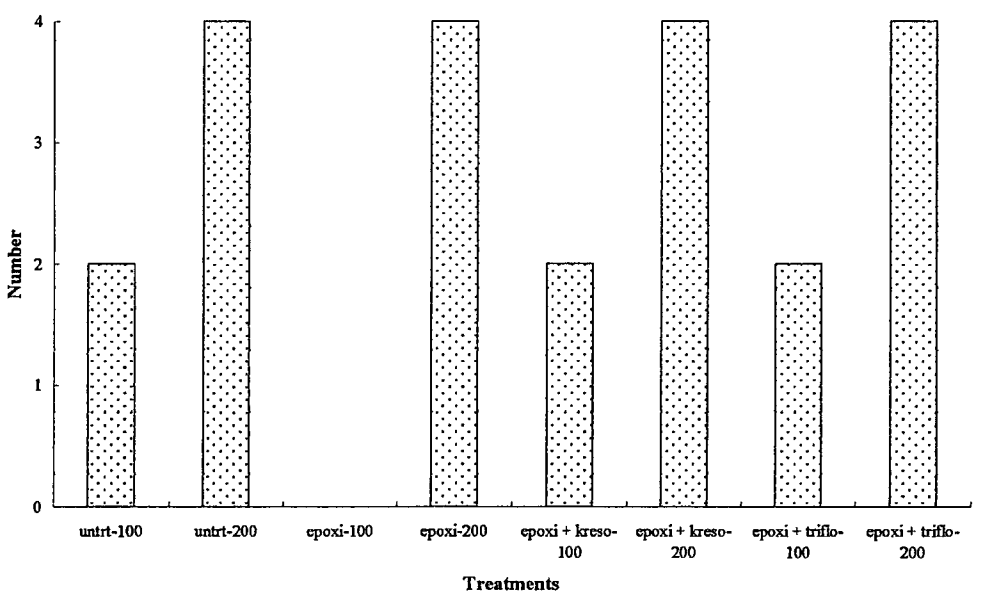
Appendix 9 (b)

The number of the plots that met the requirement of grain N concentration for bread-making (Combine-Harvested Grains) (max = 4)

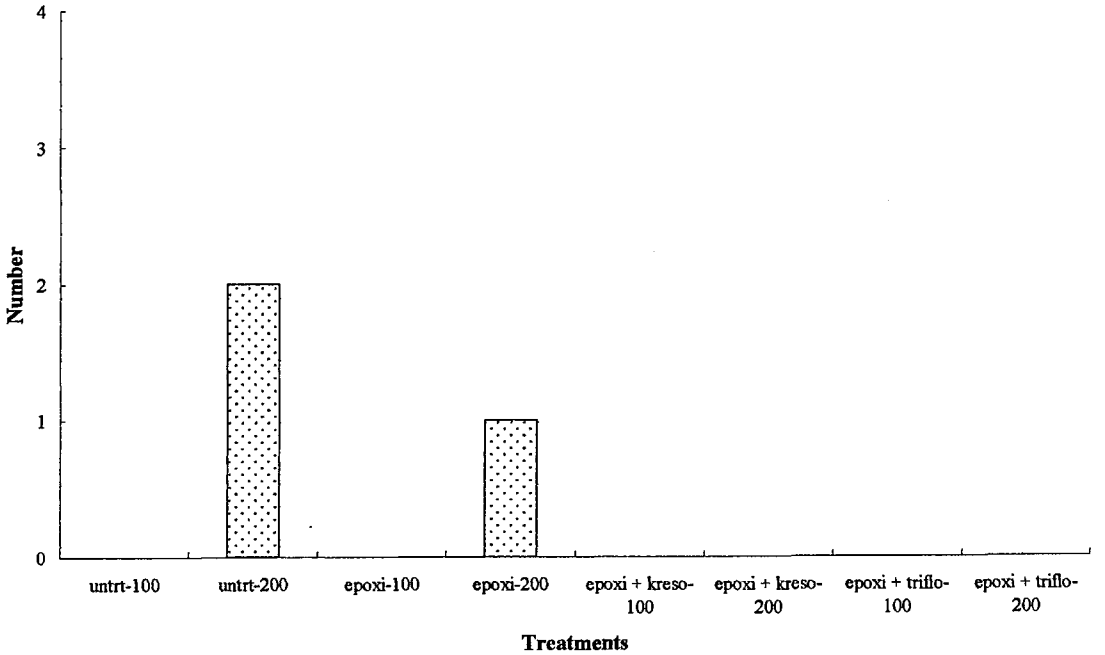
There were no plots that met the requirement in Cycle I.



(i) Cycle III-B – Hereward



(ii) Cycle III-C – Hereward



(iii) Cycle III-C – Equinox

Photograph

Photographs of the Field Experiments

Photograph 1. Cycle I Field Experiment

Photograph 2. Cycle II Field Experiment

Photograph 3. Cycle III-B Field Experiment

Photograph 4. Crop Canopy in Cycle II Field Experiment

Photograph 5. Cycle III-A Field Experiment
– Comparison of the Seed Rates of 100 m^{-2} and 400 m^{-2}

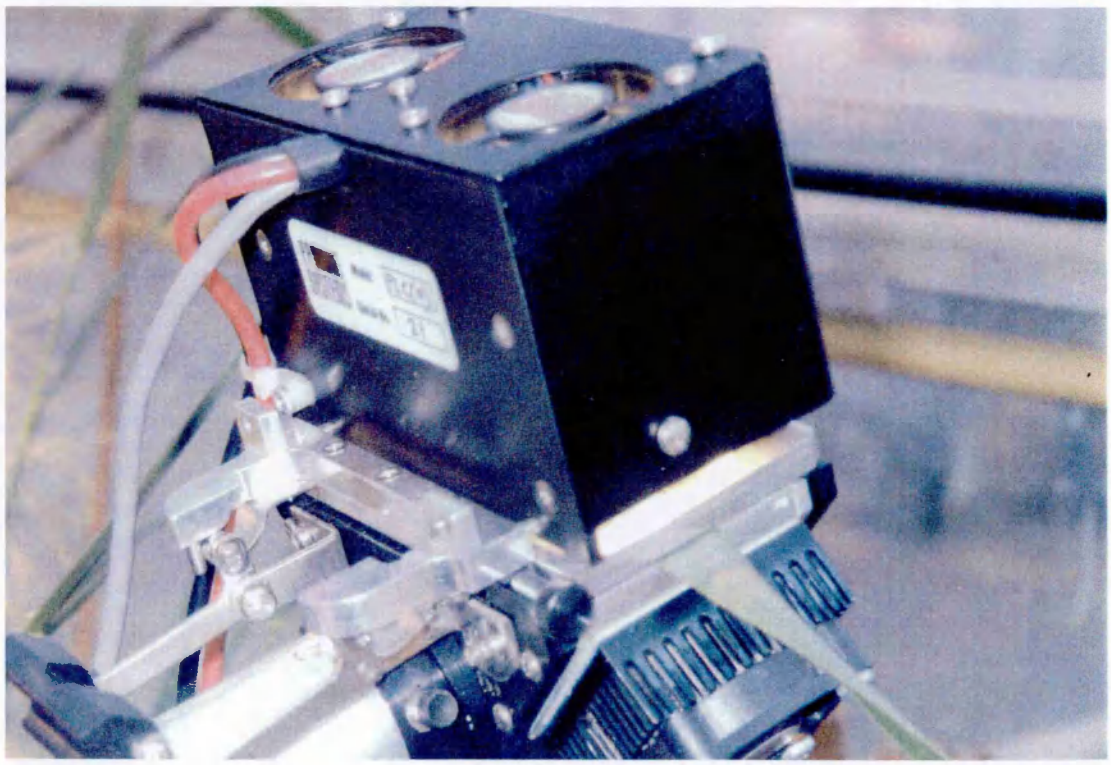
Photograph 6. 1st Sampling in Cycle III-B

Photograph 7. 5th sampling in Cycle III-B

Photograph 8. Tillers Infected by Gout Fly

Photograph 9. Tillers Infected by Gout Fly – Comparison with Healthy Tillers

Photograph 10. Infrared Gas Analyzer (IRGA) (PP Systems)



Photograph 11. Leaf Chamber for CER Measurement (PP Systems)

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